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Synthetic/Biosynthetic Phase Transfer Polymers for Pollution Minimization, Remediation, and Waste Management

Annual Report to the Office of Naval Research for

Contract N00014-93-1-0780

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Responsive Amphipathic Copolymers

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I. Objectives of the Research/Approach

The ultimate goal of this interdisciplinary proposal is the development of phase transfer polymers capable of efficient removal of water-borne pollutants from inadvertent spills or waste discharge in naval environments. Targets include petroleum based fuels and lubricants and gray water contaminants such as surfactants and oils. The polymers will be specifically tailored by synthetic or biosynthetic techniques to possess pH-responsive, hydrophobic domains which would allow capture, sequestration, and subsequent separation of foulants from water. The polymers can be recovered in the separation stage by simple pH change and can be recycled; the biopolymers proposed have the additional attribute of being biodegradable. By contrast, traditional small molecule surfactants are ineffective at high dilution for phase transfer, are virtually unrecoverable, and resist biodegradation.

Major project objectives include:

1) Preparation of amphipathic polymers and biopolymers with precisely tailored domain structures for phase transfer
2) Utilization of synthetic and biosynthetic techniques for placement of functionality for pH-responsiveness
3) Determination of structure, phase behavior, and mechanism of reversible association in aqueous media utilizing photophysical and spectroscopic techniques
4) Study of the extent and nature of transfer, sequestration, and phase stability with model hydrocarbon foulants
5) Study of the pH responsiveness for "triggered" release of foulants from associated domains
6) Study of the efficiency of polymer and model pollutant separation and recovery
7) Contribution to the interdisciplinary research and education of young scientists in biological, physical, chemical, and environmental sciences

Our approach, conceptualized in Figure 1, is to place multiple hydrophobic sequences (open circles) and ionizable moieties (squares) along a hydrophilic backbone utilizing synthetic and biosynthetic techniques. Appropriate microstructural design should allow capture of lipophilic molecules within the hydrophobic microdomains. After sequestration or domain loading, coagulation of the lipophile - rich polymer phase will allow separation from the aqueous phase. The ionizable groups built into the polymer chain could then be "triggered" by pH or electrolyte to allow lipophile (foulant) recovery and polymer redisolution for further remediation cycles.
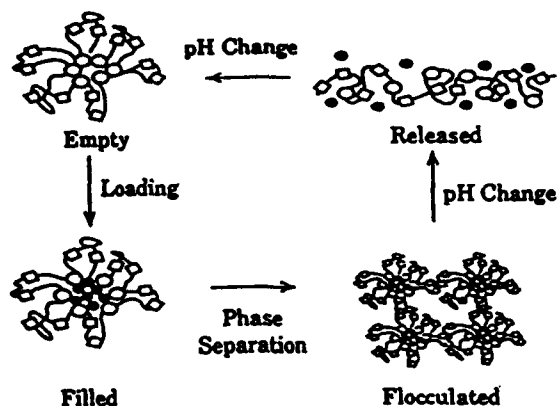
Figure 1.   Conceptual Behavior of an Amphipathic Phase Transfer Polymer in one Remediation/Recovery Cycle.

II. Major Accomplishments during year 1

Our major accomplishments have been reviewed in the following sections which include overviews of synthetic, biosynthetic, and characterization efforts. References are given to major papers, preprints, and abstracts contributed by our group within the last year. The reader is referred to those attached as an appendix to this document.

A. Synthetic Phase Transfer Polymers

Most of the recent work in our laboratories has focused on understanding and predicting the solution behavior of amphipathic polymer systems under various conditions involving pH, ionic strength, etc. The synthetic portion of this research has involved the investigation of several types of polymeric surfactants: polycations, polyanions, and polyzwitterions. Table 1 provides a list of the surfactant monomers and their corresponding acronyms that will be discussed in this report.
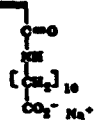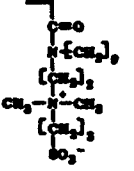
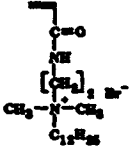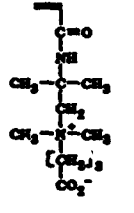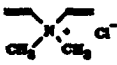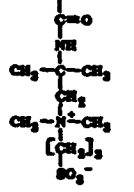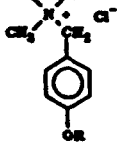| Monomer	Acronym	Monomer	Acronym	Monomer	Acronym
	AM		SA		DAATAPS
	DAMAB		AA		AMPDAB
	DADMAC		APS		AMPDAPS
	DAHMAC				

Table 1. Monomers employed and their corresponding acronyms.

A.1 Polycations

We[1] have synthesized cationic hydrophobically modified copolymers of acrylamide (AM) with dimethyldodecyl (2-acrylamidoethyl) ammonium bromide (DAMAB). Variation in the polymerization technique appears to have an effect on polymer microstructure. Three different methods of polymer synthesis were carried out. DAMAB is water-soluble and surface-active, with a critical micelle concentration (CMC) of 4.9 10^{-3} M in deionized water. The water-solubility of this monomer allows for solution copolymerization in water, and copolymerization of the two monomers in water above the CMC induces a block-like microstructure. As a radical chain end diffuses through solution, it encounters a DAMAB micelle, and most or all of the monomers in the micelle are polymerized. This leads to the formation of long runs of surface-active monomer along the polymer chain. Incorporation of a nonpolymerizable surfactant, cetyltrimethylammonium bromide (CTAB) into the monomer feed dilutes the DAMAB micellar concentration and decreases the block length. Use of a homogeneous polymerization medium such as *tert*-butanol gives a random copolymer structure.

As DAMAB content is increased, an increase in the upwards curvature in the viscosity profile is observed. DAMAB induces strong intermolecular associations via hydrophobic interactions. When copolymers of comparable DAMAB content and molecular weight, but with different microstructures are compared, block size appears to have an effect on the rheology of these systems. Overall viscosities and C* are both lower for AM/DAMAB copolymer synthesized in the presence of CTAB compared to AM/DAMAB copolymerized without added surfactant. The latter copolymer possesses a larger block length, and intermolecular associations become more favorable.

The ability of amphiphilic polymers such as AM/DAMAB to sequester hydrophobic materials in micelles formed by these systems is reflected in the emission characteristics of pyrene probe added to aqueous solutions of these copolymers. As the polarity of the environment decreases, so does the ratio of the intensity of the first vibronic band to that of the third vibronic band (I_1/I_3). I_1/I_3 decreases with increasing "blocky" polymer concentration. This suggests the presence of an intermolecular association mechanism that drives the formation of microdomains. "Random" AM/DAMAB polymer solutions exhibit concentration-independent I_1/I_3 values. This phenomenon is consistent with intramolecular hydrophobic association to form micellar domains. The low I_1/I_3 values occur at concentrations well below C*. This implies that associations occur at a microscopic level not effectively monitored by macroscopic characterization techniques such as viscosity studies.

The AM/DAMAB copolymer series also interacts with surfactants in an interesting manner.[2] The surface tension of sodium dodecyl sulfate (SDS) in the presence of AM/DAMAB copolymer is higher than in the absence of polymer. This is indicative of surfactant-polymer binding to deplete surfactant from the air-water interface. Pyrene probe studies of SDS/copolymer solutions suggest the binding of SDS to form mixed micellar SDS/DAMAB aggregates which occur below the CMC of SDS. At constant polymer concentration with increasing SDS concentration, I_1/I_3 gradually decreases, indicating non cooperative polymer-surfactant binding.

Water-soluble cyclopolymers of N,N-diallyl-N,N-dimethylammonium chloride with N,N-diallyl-N-alkoxybenzyl-N-methyl ammonium chloride (DADMAC/DAHMAC)[3] exhibit strong intramolecular associations which are broken up by the addition of SDS. As SDS concentration is increased in a solution of DADMAC/DAHMAC copolymer, a maximum in reduced viscosity is observed. This is believed to arise from interpolymer bridging by association of hydrophobic

groups from more than one polymer chain with SDS micelles. Pyrene probe studies indicate concentration-independent microdomain formation. I_1/I_3 decreases with increasing DAHMAC content, and I_1/I_3 is insensitive to copolymer concentration, which is consistent with intramolecular association.

A.2 Polyanions

Anionic hydrophobically modified copolymers can also associate in aqueous media to form hydrophobic domains. Alternating copolymers of maleic anhydride and ethyl vinyl ether that have been modified with alkyl and naphthylmethoxyheptyl groups and hydrolyzed in water form associations in water.[4] As octyl or dodecyl group substitution on the parent copolymer increases, the intrinsic viscosity in water decreases. Intramolecular hydrophobic associations collapse the polymer coil. The naphthyl label that was used to fluorescently label the copolymer is known to exhibit a polarity-dependent fluorescence lifetime (). The fluorescence lifetime of a naphthylmethoxyheptyl model compound varies from 68 nsec to 22 nsec in THF and 50/50 methanol/water, respectively. As polarity decreases, lifetime increases. In aqueous solutions of C_8 and C_{12}-modified copolymer, increases with increasing hydrophobe content. The environment experienced by naphthalene becomes more shielded from the aqueous medium due to intramolecular associations.

The ratio of naphthyl excimer to monomer emission (I_E/I_M) is shown to vary with hydrophobe content. A maximum in I_E/I_M occurs at about 30 mole % hydrophobe incorporation. As the chain collapses with increasing hydrophobe content, the separation distance between naphthyl groups on the same polymer chain decreases and the probability of dimeric excited state formation (excimer) increases. As hydrophobe content increases further, naphthalenes are "diluted" out, and domain rigidity increases. Excimer emission then becomes less favorable, and I_E/I_M decreases.

The geometry of the amphiphilic group is very crucial with respect to solution properties. The amphiphiles incorporated into the polymers discussed so far all possess "head-attached" geometries.[5,6] The ionic (or zwitterionic) group is located close to the polymer backbone, and the hydrophobe is pendent from the monomer unit. Polymerized surfactants with this geometry possess good phase transfer properties, as indicated by their ability to solubilize pyrene. The tail-attached geometry, whereby the hydrophobic tail is linked to the polymer backbone and the polar group is pendent from the monomer unit, imparts good water-solubility to polymers at the expense of domain structuring. When the hydrophobic tail is linked directly to the polymer backbone, its motion is restricted, and hydrophobic association between monomer units, whether inter- or intrapolymer, becomes unfavorable. The inhibition of hydrophobic association results in an enhancement in water-solubility.

Our research group is investigating tail-attached polymeric micelles based on acrylamide (AM) and sodium 11-acrylamidoundecanoate (SA), a novel surface-active monomer.[7] Low incorporations of SA are utilized, and small amounts alter the solution properties considerably. In deionized water, apparent viscosity increases with increasing SA incorporation. This would suggest an enhancement in polymer dimensions as hydrophobe is added. The shape of the viscosity curves does not suggest intermolecular interactions, as in typical associative polymer systems. A steady increase in viscosity with increasing polymer concentration occurs, and no upwards curvature is observed. It appears that SA incorporation imparts an open conformation to acrylamide copolymers.

As ionic strength increases, viscosity decreases. In 0.3 M sodium chloride, viscosities are considerably reduced, and increasing SA incorporation reduces the apparent viscosity. When electrostatic repulsions are shielded by salt, SA aggregates along the polymer chain collapse.

Pyrene probe studies were carried out to investigate the micellar structure of SA aggregates. I_1/I_3 decreases with increasing polymer concentration in deionized water, and a maximum is observed. The drop in I_1/I_3 values verifies the presence of hydrophobic microdomains. As polymer concentration increases, the number of SA aggregates in solution also increases, and more sites for sequestration of pyrene become available. The decrease in I_1/I_3 at higher polymer concentration suggests either a change in the number of available sites for pyrene solubilization or a change in the structure of the domains present such that pyrene is excluded.

Currently underway is an investigation of the photophysical properties of fluorescently labeled AM/SA copolymers. Incorporation of a pyrene label provides a model hydrophobe with which associations may be probed. I_E/I_M measurements indicate interpolymer association in both deionized water and sodium chloride solution at high polymer concentration. I_E/I_M studies also effectively probe coil collapse with salt or acid addition. Fluorescence quenching studies reveal that salt addition creates a constricted, less fluid microdomain.

Another anionic hydrophobically modified system which has been investigated is a labeled terpolymer composed of acrylamide (AM), acrylic acid (AA), and N-[(1-Pyrenyl-sulfonamido)ethyl]acrylamide (APS).[8] Four polyelectrolytes, denoted P2-P5 were prepared from the previously mentioned monomers by an aqueous micellar polymerization technique utilizing sodium dodecyl sulfate (SDS) to solubilize the water insoluble APS monomer. The surfactant to APS molar ratio (SMR) was varied to give a narrow range of APS monomers per micelle (n) yielding terpolymers with identical monomer compositions but varied microstructural placement of the fluorescent APS units. Terpolymer compositions are nearly identical (AM:AA:APS approximately 60:40:0.2) and conversions were kept low (<30%) to minimize terpolymer heterogeneity. After neutralization of the carboxylic acid groups, further characterization of these labeled polyelectrolytes was accomplished by viscometry, light scattering and fluorescence spectroscopy.

Weight average molecular weights (M_W) were determined by light scattering and appear to be quite similar (1.2-1.3 x 10^6 g/mole). Also, the terpolymers exhibit typical viscosity behavior for polyelectrolytes; viscosities are high in deionized water (10-12 cP at 0.02 g/dL) and decrease dramatically in 0.5M NaCl (~ 1 cP) at the same terpolymer concentration.

Steady-state fluorescence emission spectra for P2-P5 in water and in 0.5M NaCl at pH 7.0-7.5 are qualitatively identical and exhibit normal or "monomer" fluorescence from approximately 360 to 450 nm as well as excimer fluorescence from 450 to 600 nm. It is clear from the presence of excimer even at high degrees of polyelectrolyte ionization that "blocky" microstructures are evident in terpolymers P2-P5 even though very small loadings (~0.2 mole %) of the APS chromophore are present. The amount of excimer emission increases only slightly in going from water, where these labeled polyelectrolytes are in an extended conformation to 0.5M NaCl, where a more collapsed, random coil conformation is observed. The increase in excimer to monomer intensities (I_E/I_M) from deionized water to NaCl solutions indicates that long range interactions of APS chromophores do contribute to excimer formation in 0.5M NaCl, but the amount of the increase indicates that excimer formation within the terpolymer microstructure predominates. Examination of the relationship between I_E/I_M, SMR and n, indicates a number of important factors concerning the micellar polymerization technique. Decreasing the SMR in

the polymerization feed increases the initial number of APS monomers per micelle which in turn affects the label or hydrophobe proximity in the resulting terpolymers. In fact, a linear relationship holds between I_E/I_M and n and implies that the "initial conditions" in the polymerization feed controls the microstructure of the resulting terpolymers. Control of polymer microstructure and the effects of hydrophobe sequence distribution on associative properties and domain-forming ability is the subject of an upcoming publication. Future studies in this area will focus on the effects of increased label incorporation in the domain-forming ability of similar systems as well as the interplay of polyelectrolyte charge density on aggregation and sequestration.

A.3 Polyzwitterions

Previous work in our labratories dealt with the synthesis of polyampholytes which contain a zwiterionic mer unit that possesses a quaternary ammonium moiety as the cationic group and a sulfonate moiety as the anionic group. In an effort to synthesize polymers which dispaly both polyelectrolyte and polyampholyte behavior depending on solution pH as well as electrolyte content, terpolymers of acrylic acid (AA), acrylamide (AM), and the zwitterionic monomer 3-[(2-acrylamido-2-methylpropyl)dimethylammonio]-1-propanesulfonate (AMPDAPS) have been prepared by free radical polymerization in a 0.5M NaCl aqueous solution using potassium persulfate as the initiator.[9] The feed ratio of AMPDAPS:AA:AM was varied from 5:5:90 to 40:40:20 mol %, with the total monomer concentration held constant at 0.45M. Terpolymer compositions were obtained by ^{13}C NMR and indicated a preferential incorporation of the acrylamido type monomers in the terpolymer. Low angle laser light scattering provided molecular weights and second virial coefficients which varied from (3.0 to 7.9) x 10^6 and (2.23 to 2.95) x 10^{-4} mL mol g^{-2}, respectively. The solubilities of the resulting terpolymers are dependent on pH as well as the amount of AMPDAPS and AA present in the feed. At pH=4 and for higher incorporation of AA and AMPDAPS in the feed (>25 mol%), the terpolymers are insoluble in deionized water and 0.25M NaCl. This behavior is a result of hydrogen bonding between the acrylic acid and acrylamide mer units and electrostaic attractions between the AMPDAPS mer units. At pH=8, all terpolymers are soluble in deionized water and salt solutions as a result of neutralization of the carboxylic acid groups to carboxylate groups. The dilute and semidilute solution behaviors of the terpolymers were studied as a function of composition and added electrolytes. Polyelectrolyte behavior was observed for all terpolymers at pH 8 as evidenced by a viscosity decrease in the presence of added electrolytes. The terpolymers exhibit higher viscosities in the presence of NaSCN versus NaCl. Comparison of the solution behavior of the terpolymers to copolymers of AM and AA as well as copolymers of AMPDAPS and AM has been made. In most instances, the solution viscosity behavior of the terpolymers was superior to that of the copolymers.

In continuation of the synthesis of pH responsive polyampholytes, we are currently investigating the solution behavior of copolymers of acrylamide (AM) and 4-(2-acrylamido-2-methylpropanedimethylammonio) butyric acid (AMPDAB).[10] This series of copolymers contains a zwitterionic mer unit that possesses a carboxylate group as the anionic moiety. The carboxylate group was chosen due to the ability of this moiety to be protonated. This allows these systems to behave as polycations or polyampholytes depending on the pH of the aqueous media. The feed ratio of AM:AMPDAB was varied from 90:10 to 75:25 mol%. Reactivity ratios were determined and indicated random comonomer incorporation into the polymer implying similar reactivity for the two monomers. As anticipated, apparent viscosities at pH=3 were high,

indicative of the polycationic nature of the polymer. At low pH values, the carboxylate group located on the AMPDAB mer unit becomes protonated and the polymer acquires an overall positive charge. The resulting cationic groups repel one another and the polymer chain adopts a more extended conformation. Intrinsic viscosities were determined for low incorporations of the AMPDAB mer unit (<25 mol%) and for the homopolymer of AMPDAB in varying ionic strengths of NaCl at pH=8. At pH=8, the AMPDAB mer units are in the zwitterionic state. The copolymers containing a low incorporation of AMPDAB exhibited complex solution behavior. At low ionic strengths, there is an initial decrease in the intrinsic viscosity likely due to the elimination of intermolecular interactions. After a critical concentration of NaCl is added, the polymers display typical polyampholyte behavior as evidenced by an increase in intrinisic viscosity as intramolecular interactions are reduced. The homopolymer exibited an increase in intrinsic viscosity as a function of increasing NaCl concentration, indicating the shielding of intramolecular attractions. Further work with these systems is to investigate how changing the number of methylene units between the ammonium group and the carboxylate group affects the solution behavior.

Copolymers of acrylamide (AM) and 3-(N,N-dimethyl-N-3'-(N'-acryloyl)aza-tridecyl) ammonion propane sulphonate (DAATAPS)[11] have also been synthesized in our laboratories. These polymerizations were carried out in aqueous media. Due to the insolubility of the copolymer, only a 1% hydrophobe incorporation could be attained. The polymerization was carried out in the presence of varying amounts (0, 25, 50, 75 mole%) of nonpolymerizable surfactant (SDS) in order to study any effect the polymerization technique may have on the polymer microstructure. Through viscosity studies, it was confirmed that solution salinity had no effect on this property. A linear increase in viscosity was observed from 1.19-1.84 cP for polymer concentrations of 0.10-0.40 g/dL under conditions of 0M NaCl, 0.051M NaCl, and 0.26M NaCl. Also through viscosity studies, it was observed that an increase in SDS concentration during polymerization produces a decrease in viscosity, implying from the work mentioned above that microstructure blockiness increases with decreasing nonpolymerizable surfactant concentration. Currently underway is the synthesis of the carboxylate analog of DAATAPS.

II. B. Biosynthetic Phase Transfer Polymers

B.1 Naturally Occurring Proteins

Much progress has been made in the past year towards achieving our goals of designing and producing responsive biopolymers. In both the areas of naturally occurring proteins and designed polypeptides, we have been able to synthesize or isolate, partially purify, and to some extent, characterize biopolymers which possess attributes required for surface activity and stable phase partitioning of hydrocarbons in aqueous environments.

In the area of naturally occurring polypeptides, we focused our attention primarily on two types of proteins, the oleosins from the soybean, Glycine *max*, and Apolipophorin-III (Apolp-III) from the tobacco hornworm, *Manduca sexta*. Both of these proteins are amphipathic in nature and *in vivo*, associate with hydrophobic particles. The oleosins are oil body membrane proteins found in seeds. Several of these proteins function to stabilize an oil body by embedding in the phospholipid monolayer of the particle. The association of these proteins with phospholipid/triacylglycerol (TAG) oil bodies prevents the coalescence of these particles via hydrophobic interactions of the proteins with the nonpolar side chains of the lipids and the TAG.[12] Because these molecules can interact with hydrophobic particles to stabilize them, they offer promise as possible phase transfer agents for hydrocarbons in aqueous solutions.

The soybean oleosins have been extracted from germinating seeds according to published methods.[13] Denaturing polyacrylamide gel electrophoresis (SDS-PAGE) revealed 4 bands of 17, 18, 24, and 34 kDa.[14] Ammonium sulfate precipitation, Triton X-114 phase separation, affinity chromatography with a phenyl sepharose column, gel filtration chromatography (S-200 column) and non denaturing polyacrylamide gel electrophoresis did not separate the proteins. However, the presence of only one chromatographic peak on S-200 and a sole band on a native polyacrylamide gel, both of high molecular weight, suggested that the individual oleosin proteins were associating to form a supramolecular assembly. This was confirmed by quasielastic light scattering which also indicated protein particles of large sizes.[15,16]

Preliminary interfacial tension measurements were conducted to evaluate the efficiency of the soybean oleosins as amphipathic molecules. These measurements were performed at a cyclohexane/water interface to which protein was added. At pH 2.6 and 11.5, the interfacial activity of the protein containing samples was substantially higher than that of the samples at the natural pH of the oleosin suspension, pH 5.2. This behavior is believed to be due to the decreased solubility of the proteins at pH 5.2 and at extreme pH, to the chain expansion by like charge repulsion of the proteins to expose their hydrophobic portions. These studies indicate that the interfacial activity of the oleosins is pH responsive, with the greatest reduction of interfacial tension at low pH.[17,18,19]

Like the oleosins from *Glycine max*, the Apolipophorin-III from *Manduca sexta* is an amphipathic protein. *In vivo*, it associates with lipophorin particles, composed of phospholipids and diacylglycerols, to allow them to be stably transported through the aqueous hemolymph of the insect. Instead of isolating this protein from the insect, we have chosen to recombinantly produce the Apolp-III in *E. coli* to facilitate production of large amounts of protein. In addition, recombinant production of Apolp-III should also enable site directed mutagenesis on the gene and hence, the protein. Mutations in the protein sequence should allow for the design of controlled responsive behavior into Apolp-III.

Apolp-III, including its signal peptide, has been cloned and recombinantly expressed as a fusion protein.[20] This fused protein has been chromatographically purified and analyzed by SDS-PAGE and immunoblotting.[21] As purification of Apolp-III from the fusion proved to be difficult, another means of expressing the protein from a construct in a Novagen pET vector was explored. The protein expressed from this construct appeared in the soluble cell fraction and was determined to be the correct protein by SDS-PAGE and immunoblotting.[22,23] Attempts are currently being made to clone and express the gene, without its signal peptide, in a pET construct. The polymerase chain reaction is being used to isolate and mutagenize the gene.

B.2 de novo Polypeptides

An amphipathic a-helical polypeptide, DN3L, has been designed and recombinantly cloned and expressed. The design of this polypeptide was based on secondary structures of naturally occurring proteins which associate with hydrophobic materials. The design also included a flexible, acidic hinge to permit control over the associative properties of DN3L.[24]

DN3L has been purified from whole cell E. coli lysates to 99.9% purity. The first eleven amino acids of DN3L have been confirmed by peptide sequencing. At pH 7.6 this peptide migrates as two bands on an SDS-PAGE gel. The molecular weights of these bands are 3,750 and 14,400 Daltons. These molecular weights correspond to the DN3L monomer and an a DN3L aggregate containing four molecules. The aggregate appears to be very stable, resisting denaturation when boiled in either a 1.2% SDS solution or 8.0 M urea.[25] Laser desorption mass spectroscopy detects a major species and three minor ones. The primary species, of molecular weight 3,720 Daltons, is the DN3L monomer. The remaining three species have been identified as dimer, trimer and tetramer aggregates of DN3L. FTIR studies of DN3L in D_2O indicate the presence of a-helical structures.

The associative properties of DN3L appear to be pH dependent. Acidified samples of DN3L migrate as three bands on an SDS-PAGE gel. The molecular weight of these bands correspond to a monomer, dimer and tetramer of DN3L.[26] A qualitative change in surface behavior has been observed in acidified samples of DN3L. Initial surface tension studies at physiological pH show a decrease in surface tension from 70 mN/m to 56 mN/m with increasing protein concentration. Surface tension studies at low and high pH are currently underway.

Preliminary pyrene fluorescence probe experiments have indicated the presence of pH stable, nonpolar microdomains in solutions containing DN3L. Additional fluorescence studies using 1-anilino-8-naphthalene sulfonate (ANS), as well as studies with quantitative circular dichroism, are in progress.

III. Potential Benefits

Increasingly strict regulations, growing water utilization, and responsible environmental stewardship are all reasons for development of new water remediation technologies. In this research we have begun an interdisciplinary program involving synthesis, molecular characterization, and phase studies to develop amphipathic synthetic and biosynthetic polymers which circumvent some of the problems associated with conventional approaches to remediation. It is anticipated that polymers forthcoming from this effort could ultimately be utilized in remediation of water in confined or nonconfined environments. Targeted for initial treatment were waters contaminated with petroleum based hydrocarbons, lubricants, fuels toxic organic chemicals, and amphiphilic materials in gray water discharge.

Discoveries during the first year of this work of strong polymer-surfactant binding presents the intriguing prospect of surfactant removal by responsive amphipathic polymers. This aspect will be pursued in future work. Additionally, we have initiated an effort in concert with other projects within the ONR remediation program aimed at incorporating promising candidate polymers into nano-composite membranes. Reversible (self cleaning) membranes might be envisioned utilizing concepts from this work.

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15. Richardson, M. F.; McCormick, C. "Design of a Novel Phase Transfer Polypeptide Based on Solution Studies of Naturally Occurring Amphipathic Proteins," *Abstracts* Sixth Annual Southeastern Graduate Polymer Conference, University of Southern Mississippi, April 13, **1994**.

16. Richardson, M. F.; McCormick, C. "Interaction of Apolipophorin-III from *Manduca sexta* and Oleosin from *Glycine Max* in Water in the Presence of Hydrophobic Materials," National ACS Meeting, Washington, DC, August **1994**.

17. Lochhead, R.; Tisack, M. "An Investigation of Plant Oleosins as Polymeric Emulsifiers," *Abstracts* Sixth Annual Southeastern Graduate Polymer Conference, University of Southern Mississippi, April 13, **1994**.

18. Lochhead, R.; Tisack, M. "An Investigation of Plant Oleosins as Polymeric Emulsifiers," Society of Cosmetic Chemists Meeting, Las Vegas, Nevada, May 6, **1994**.

19. Lochhead, R.; Tisack, M. "An Investigation of Plant Oleosins as Polyₑ ᵣic Emulsifiers" ACS 68th Colloid and Surface Science Symposium, Stanford, California, June 19, **1994**.

20. Kahalley, J.; Heinhorst, S.; Cannon, G.; McCormick, C. L. "Responsive Biopolymers for Water Remediation via Phase Transfer Interactions," Southeastern ACS meeting, Johnson City, TN, October, **1993**.

21. Kahalley, J.; Cannon, G.; McCormick, C. L. "Recombinant Synthesis of *Manduca sexta* Apolipophorin-III: A Protein for Phase Transfer of Hydrocarbons from Aqueous Environments," *Abstracts MAS*, p.34, Biloxi, MS, February **1994**.

22. Kahalley, J.; Cannon, G.; McCormick, C. " Cloning and Expression of *Manduca sexta* Apolipophorin-III: A Model Protein for Removal of Hydrophobic Contaminants from Water," *Abstracts* Sixth Annual Southeastern Graduate Polymer Conference, University of Southern Mississippi, April 13, **1994**.

23. Kahalley, J.; Cannon, G.; McCormick, C. L. "Cloning, Expression, and Purification of Recombinant Apolipophorin-III: A Biopolymer for Phase Transfer of Hydrocarbons from Aqueous Environments," National ACS Meeting, Washington, DC, August **1994**.

24. Logan, M.; Cannon, G.; Heinhorst, S.; McCormick, C. "Synthesis and Design of *de Novo* Hydrophobically Associating Polypeptides," *Polymer Preprints, 34(1)*, 140 (**1993**).

25. Logan, M.; Cannon, G.; Heinhorst, S.; McCormick, C. "Design and Synthesis of a *de novo* Hydrophobically Associating Polypeptide for Phase Transfer Applications," Southeastern ACS meeting, Johnson City, TN, October, **1993**.

26. Logan, M.; Cannon, G.; McCormick, C. "The Use of Recombinant DNA Technology to Produce Water Soluble Polymers," *Abstracts* Sixth Annual Southeastern Graduate Polymer Conference, University of Southern Mississippi, April 13, **1994**.

Appendix

Office of Naval Research
Publications/Patents/Presentations/Honors Report

R&T Number:
Contract/Grant Number   N00014-93-1-0780
Contract/Grant Title: Synthetic/Biosynthetic Phase Transfer
                                         Polymers for Pollution Minimization,
                                         Remediation, and Waste Management
Principal Investigator: Charles L. McCormick
Mailing Address: Southern Station Box 10076
                                         Hattiesburg, MS 39406-0076
Phone Number: (601) 266-4872 Fax Number: (601) 266-5504

a. Number of papers submitted to refereed journals, but not published: 6
b. Number of papers published in refereed journals, (list attached): 8
c. Number of books or chapters submitted, but not yet published: 0
d. Number of books or chapters published (list attached): 0
e. Number of preprints: 4
f. Number of patents filed: 0
g. Number of patents granted: 0
h. Number of invited presentations at workshops/professional mettings: 0
i. Number of presentations at workshops or professional meetings: 5
j. Honors/Awards/Prized for contract/grant employees: 0
k. Total number of Graduate Students and Post-Doctoral associates supported
    by at least 25% during this period, under this R&T project number:
        Graduate Students 11
        Post-Doctoral Associates 2
    including the number of,
        Female graduate students 4
        Minority Graduate students 0
        Asian Graduate Students 1
l. Other funding (list attached)

A.   Technical Papers Submitted

"Water Soluble Copolymers. 53: ^{23}Na NMR Studies of Hydrophobically-Modified Polyacids: Copolymers of 2-(1-Napthylacetamido)-ethylacrylamide with Acrylic acid and Methacrylic acid," J. Kent Newman and C. L. McCormick, submitted to *Macromolecules* (May 1993).

"Water Soluble Copolymers 52: ^{23}Na NMR Studies of Ion-Binding to Anionic Polyelectrolytes: Poly(sodium 2-acrylamido-2-mehtylpropanesulfonate), Poly(sodium 3-acrylamido-3-methylbutanoate), Poly(sodium acrylate) and Poly(sodium galacturonate)," J. Kent Newman and C. L. McCormick, submitted to *Macromolecules* (May 1993).

"Water Soluble Copolymers: 54. N-isopropylacrylamide-co-acrylamide Copolymers in Drag Reduction: Synthesis, Characterization and Dilute Solution Behavior," Pavneet S. Mumick and Charles L. McCormick, submitted to *Polymer Science and Engineering* (May 1993).

"Water Soluble Copolymers: 55. N-isopropylacrylamide-co-acrylamide Copolymers in Drag Reduction: Effect of Molecular Structure, Hydration and Flow Geometry on Drag Reduction, Performance, Pavneet S. Mumick, Roger D. Hester and Charles L. McCormick, submitted to *Polymer Science and Engineering* (May 1993).

"Water Soluble Copolymers. 57. Amphiphilic Cyclocopolymers of Diallylalkoxybenzylmethylammonium Chloride and Diallyldimethylammonium Chloride," Yihua Chang and Charles L. McCormick, submitted to *Polymer* (October, 1993).

"Water Soluble Co-Polymers 59. Investigation of the Effects of Polymer Microstructure on the Associative Behavior of Amphiphilic Terpolymers of Acrylamide, Acrylic Acid and N-[(4-decyl)phenyl]acrlyamide," K. D. Branham, D. L. Davis, J. C. Middleton and Charles L. McCormick, submitted to *Polymer* (September, 1993).

B. Technical Papers Published

Water Soluble Copolymers. 47. Copolymerization of Maleic Anhydride and N-Vinylformamide: Charge Transfer Complexation of the Monomers and Their Reactivity Ratios," Y. Chang and C. L. McCormick, *Macromolecules 26*, 4814-4817 (1993).

"Water Soluble Copolymers. 48. Reactivity Ratios of N-vinylformamide with Acrylamide, Sodium Acrylate, and n-Butyl Acrylate," Erich E.L. Kathmann and C. L. McCormick, *Macromolecules 26, 52-49-5252* (1993).

"Water Soluble Copolymers. 49. Effect of the Distribution of the Hydrophobic Cationic Monomer DAMAB on Solution Behavior of Associating Acrylamide Copolymers," Y. Chang and C. L. McCormick, *Macromolecules 26, 6121-6126* (1993).

"Water Soluble Co-Polymers 50: Effect of Surfactants on the Solution Properties of Amphipathic Copolymers of Acrylamide and N,N-Dimethyl-N-dodecyl-N-(2-acrylamidoethyl)ammonium Bromide," Yihua Chang, Robert Y. Lochhead, and Charles L. McCormick, *Macromolecules, 27*, 2145-2150 (1994).

"Water Soluble Copolymers LI: Copolymer Compositions of High-Molecular Weight Functional Acrylamido Water-Soluble Polymers Using Direct Polarization Magic-Angle Spinning ^{13}C NMR," J. Kent Newman and C. L. McCormick, *Polymer* 35, 935-938 (1994).
"Water Soluble Copolymers: 56. Structure and Solvation Effects of Polyampholytes in Drag reduction," Pavneet S. Mumick, Paul M. Welch, Luis C. Salazar and Charles L. McCormick, *Macromolecules* 27, 323-331 (1994).

"Water Soluble Co-Polymers 58: Associative Interactions and Photophysical Behavior of Terpolymers Prepared by Modification of Maleic Anhydride/Ethyl Vinyl Ether Copolymers," Yihua Chang and Charles L. McCormick, *Macromolecules, 27*, 2151-2158 (1994).

"Water Soluble Co-Polymers 60. Synthesis and Soluiton Behavior of Terpolymers of Acrylic Acid, Acrylamide, and the Zwitterionic monomers 3-(2-Acrylamideo-2-methylpropanedimethylammonio)-1-propanesulfonate," *Macromolecules, 27(12)*, pp.3156-3161 (1994).

C. Preprints

"The Use of Recombinant DNA Technology to Produce Water Soluble Polymers," M. Logan, G. Cannon, and Charles L. McCormick, *Abstracts* Sixth Annual Southeaster Graduate Polymer Conference, University of Southern Mississippi, April 13, 1994.

"Synthesis and Design of *Denovo* Hydrophobically Associating Polypeptides," M. Logan, G. Cannon, S. Heinhorst, and C. McCormick, *Polymer Preprints, 34(1)*, 140 (1993).

"Rheological and Photophysical Investigation of Domain-Forming Hydrophobic Polyelectrolytes Based on Sodium 11-Acrylamidoundecanoate," M. C. Kramer, C. G. Farmer-Welch and C. L. McCormick, *Polymer Preprints, 34(1)*, 999 (1993).

"Cloning and Expression of *Manduca sexta* Apolipophorin-III: A Model Protein for Removal of Hydrophobic Contaminants from Water, J. Kahalley and Charles L. McCormick, *Abstracts* Sixth Annual Southeastern Graduate Polymer Conference, University of Southern Mississippi, April 13, 1994.

D. Presentations

"Synthesis and Design of *Denovo* Hydrophobically Associating Polypeptides," M. Logan, G. Cannon, S. Heinhorst, and C. McCormick, National ACS Meeting, Denver, CO, March 1993.

"Derivatization of Chitin and Cellulose Utilizing LiCl/N,N-Dimethylacetamide Solvent System," S. L. Williamson and C. L. McCormick, National ACS Meeting, Denver, CO, March 1993.

"Controlled Activity Polymers: Synthesis, Characterization and Reactivity Ratios of β-Naphthyl Acrylate, 5-Acrylamido(β-Naphthyl)Valerate, and 6-Acrylamido(β-Naphthyl) Caproate Copolymers," C. Boudreaux, D. Sellers and C. L. McCormick, National ACS Meeting, Denver, CO, March 1993.

"Effect of Surfactants on the Solution Properties of Hydrophobically Modified, Cationic Polyacrylamides," Y. Chang and C. L. McCormick, National ACS Meeting, Denver, CO, March 1993.

"Rheological and Photophysical Investigation of Domain-Forming Hydrophobic Polyelectrolytes Based on Sodium 11-Acrylamidoundecanoate," M. C. Kramer, C. G. Farmer-Welch and C. L. McCormick, National ACS Meeting, Denver, CO, March 1993.

"^{23}Na NMR Studies of Ion-Binding to Anionic Polyelectrolytes: Sodium 2-Acrylamido-2-Methylpropanesulfonate (NaAMPS) and Sodium 3-Acrylamido-3-Methylbutaneoic Acid (NaAMB)," J. Kahalley, J. K. Newman and C. L. McCormick, National ACS Meeting, Denver, CO, March 1993.

"Synthesis and Solution Behavior of Polyelectrolyte/Polyampholyte Terpolymers Based on 3-(2-Acrylamido-2 methylpropanedimethylammonio)-1-propanesulfonate, Acrylic Acid, and Acrylamide," E. E. L. Kathmann, D. L. Davis and C. L. McCormick, National ACS Meeting, Denver, CO, March 1993.

"Synthesis and Aqueous Solution Properties of Responsive Polyelectrolytes and Polyampholytes," C. L. McCormick, M. C. Kramer, Y. Chang, K. D. Branham, and E. L. Kathmann, National ACS Meeting, Denver, CO, March 1993.

"Tailored Copolymer Structures: Effects on Drag Reduction in Aqueous Media," C. L. McCormick and P. S. Mumick, National ACS Meeting, Denver, CO, March 1993.

"A New Generation of Electrolyte and pH Responsive Water-Soluble Polymers for Mobility Control," C. L. McCormick, K. D. Branham, D. L. Davis, and J. C. Middleton, National ACS Meeting, Petroleum Chemistry Division, Denver, CO, March 1993.

"Hydrophobically-Modified Polyelectrolytes: Rheological Properties in Aqueous Solution," E. Kathmann, Y. Chang, and C. L. McCormick, National ACS Meeting, Polyelectrolytes Symposium, Denver, CO, March 1993.

"Synthesis and Solution Characterization of Cationic, Hydrophobically Modified Acrylamide Copolymers," Y. Chang and C. L. McCormick, ANTEC, New Orleans, LA, May 1993.

"Investigation of Domain-Forming Hydrophobic Polyelectrolytes Via Rheological nad Photophyscial Studies," M.C. Kramer, C. L. McCormick, 5th Annual Graudate Polymer Conference Georgia Tech., Univ. April 1993.

"Structure/Property Relationships in Associative Polyelectrolytes: Effects of Synthetic Parameters on Polymer Microstructure and Solution Properties," K.D. Branham, G. L. Shafer, D. L. Davis, and C. L. McCormick, 5th Annual Graudate Polymer Conference Georgia Tech., Univ. April 1993.

"Responsive Biopolymers for Water Remediation via Phase Transfer Interactions," Johanna M. Kahalley, Sabine Heinhorst, G. C. Cannon, and C. L. McCormick, Southeastern ACS meeting, Johnson City, TN, October 1993.

"Design and Synthesis of a de novo Hydrophobically Associating Polypeptide for Phase Transfer Applications," Mark J. Logan, Gordon C. Cannon, Sabine Heinhorst, and C. L. McCormick, Southeastern ACS meeting, Johnson City, TN, October 1993.

"Synthesis and Solution Properties of Cyclocopolymers of Alkyldiallylmethylammonium Chloride and Diallyl-dimethylammonium Chloride," Yihua Chang and C. L. McCormick, Southeastern ACS meeting, Johnson City, TN, October 1993.

"Controlled Activity Polymers: Synthesis, Characterization, and Reactivity Ratios of 3-acrylamido-3-methyl(β-naphthyl)butanoate, and 2-acrylamido(β-naphthyl)valerate Copolymers," Chase Boudreaux and C. L. McCormick, Southeastern ACS meeting, Johnson City, TN, October 1993.

"Synthesis and Characterization of a Water-soluble Polyampholyte Based on a Novel Zwitterionic Betaine Monomer, E.E. Kathmann and C.L. McCormick, Southeastern ACS meeting, Johnson City, TN, October 1993.

"Design of Anionic Polymeric Surfactants Containing Nonionic Hydrophobic Groups for Enhanced pH and Salt-responsiveness," Michael C. Kramer and C. L. McCormick, Southeastern ACS meeting, Johnson City, TN, October 1993.

"Derivatization of Chitin and Cellulose Utilizing Licl/n,n-dimethyl Acetamide," Sheila Williamson and C. L. McCormick, Southeastern ACS meeting, Johnson City, TN, October 1993.

"The Synthesis and Characterization of Novel Hydrophobically Modified Zwitterionic Copolymers," Katherine M. Johnson and C. L. McCormick, Southeastern ACS meeting, Johnson City, TN, October 1993.

"Determination of Terpolymer Microstructure via Fluorescence Studies in Dilute Solution," Kelly D. Branham, Georgia L. Shafer, and C. L. McCormick, Southeastern ACS meeting, Johnson City, TN, October 1993.

"Recombinant Synthesis of Manduca Sexta Apolipophorin-III: A Protein for Phase Transfer of Hydrocarbons from Aqueous Environments," Johanna Kahalley, Gordon Cannon, and Charles L. McCormick, *Abstracts MAS*, p. 34, Biloxi, MS, February 1994.

"Photophysical Studies of Hydrophobically Modified Anionic Polymeric Surfactants," Jamie R. Steger, Michael C. Kramer, and Charles L. McCormick, *Abstracts MAS*, p. 45, Biloxi, MS, February 1994.

"Controlled Activity Polymers: Synthesis, Characterization and Controlled Release Studies of Copolymers Designed for the Hydrolytic Release of β-Napthol," Chase Boudreaux, Clay Bunyard, and Charles L. McCormick, *Abstracts MAS*, p. 46, Biloxi, MS, February 1994.

"Biosurfactant Properties of Natural and Recombinantly Modified Soybean Oleosin," Jianbin Zue, Charles L. McCormick, and Gordon C. Cannon, *Abstracts MAS*, p. 34, Biloxi, MS, February 1994.

"A Study of Micellar Polymerization by Photophysical Methods: Variation of Label Proximity with Surfactant Concentration," K. D. Branham and Charles L. McCormick, *Abstracts* Sixth Annual Southeastern Graduate Polymer Conference, University of Southern Mississippi, April 13, 1994.

"Controlled Activity Polymers: Synthesis and Characterization of β-Naphthyl Acrylate, 5-Acrylamideo (β-Naphthyl)Valerate, 2-Acrylamido(β-Naphthyl)Valerate,

3-Acrylamido-3-Methyl-(β-Naphthyl)Butanoate, and 6-Acrylamido(β-Naphthyl)Caproate with Acrylic Acid," Chase Boudreaux and Charles L. McCormick, *Abstracts* Sixth Annual Southeastern Graduate Polymer Conference, University of Southern Mississippi, April 13, 1994.

"The Synthesis and Characterization of Novel Hydrophobically Modified Zwitterionic Copolymers," K. M. Johnson and Charles L. McCormick, *Abstracts* Sixth Annual Southeastern Graduate Polymer Conference, University of Southern Mississippi, April 13, 1994.

"Cloning and Expression of *Manduca sexta* Apolipophorin-III: A Model Protein for Removal of Hydrophobic Contaminants from Water, J. Kahalley and Charles L. McCormick, *Abstracts* Sixth Annual Southeastern Graduate Polymer Conference, University of Southern Mississippi, April 13, 1994.

"Hydrophillic Carboxybetaine Copolymers of Acrylamide and 4-(2-Acrylamido-2-Methylpropyldimethylammonio) Butyric Acid," *Abstracts* Sixth Annual Southeastern Graduate Polymer Conference, University of Southern Mississippi, April 13, 1994.

"Fluorescence Studies of Pyrene-Labeled Water-Soluble Polyelectrolytes," M. C. Kramer and Charles L. McCormick, *Abstracts* Sixth Annual Southeaster Graduate Polymer Conference, University of Southern Mississippi, April 13, 1994.

"The Use of Recombinant DNA Technology to Produce Water Soluble Polymers," M. Logan, G. Cannon, and Charles L. McCormick, *Abstracts* Sixth Annual Southeastern Graduate Polymer Conference, University of Southern Mississippi, April 13, 1994.

"Design of a Navel Phase Transfer Polypeptide Based on Solution Studies of Naturally Occurring Amphipathic Proteins," M. F. Richardson and Charles L. McCormick, *Abstracts* Sixth Annual Southeastern Graduate Polymer Conference, University of Southern Mississippi, April 13, 1994.

"Derivatization of Chitin and Cellulose Utilizing LicL/N,N-Dimethyl Acetamide," Sheila Williamson and Charles L. McCormick, *Abstracts* Sixth Annual Southeastern Graduate Polymer Conference, University of Southern Mississippi, April 13, 1994.

"Advances in Synthetic Water Soluble Polymers," C.L. McCormick, 1994 High Polymer Research Conference, Moretonhampstead, Devon, England, April 25-29, 1994.

I. Other Funding

DOE/EPSCoR Traineeship," DOE/EPSCoR, $15,625/year (1992-1994)

"Responsive Copolymers for Enhanced Oil Recovery," Department of Energy, $295,002 (1993).

Unilever Research Fellowship, $15,000 (1993).

Exxon Chemical Company, $10,000 (1993).

Gillette Research Institute, $15,000 (1993).

Mobile Oil Company, $10,000 (1993)

Biopolymer Development, USM, $42,000 (1993)

DOD/ONR, Synthetic/Biosynthetic Phase Transfer Polymers for Pollution Minimization, Remediation, Waste Management — from Office of Naval Research 3 yr. Grand Total $788,966 -- $250,257 (1993)

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Water-Soluble Copolymers. 49. Effect of the Distribution of the Hydrophobic Cationic Monomer Dimethyldodecyl(2-acrylamidoethyl)ammonium Bromide on the Solution Behavior of Associating Acrylamide Copolymers

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ABSTRACT: A novel water-soluble monomer, dimethyldodecyl(2-acrylamidoethyl)ammonium bromide (DAMAB), was synthesized. This monomer possesses a critical micelle concentration of 4.9×10^{-3} M. A series of copolymers of DAMAB with acrylamide (AM) have been prepared by radical copolymerization by micellar and solution techniques. The rheological properties of the copolymers were strongly affected by their microstructures. A random copolymer with 5 mol % of DAMAB obtained by solution polymerization in *tert*-butyl alcohol showed a tendency for intramolecular hydrophobic association, while microheterogeneous copolymerization of AM with 5 and 10 mol % of DAMAB in water yielded microblocky structures which promoted intermolecular association of hydrophobes. The intermolecular association was enhanced by increasing the length of the hydrophobic block and/or the number of blocks in the polymer chain. Evidence of hydrophobic microdomains was obtained utilizing pyrene probe fluorescence.

Introduction

Hydrophobically modified polymers have been the subject of considerable research beginning with the classical studies of Strauss.[1] These amphipathic polymers exhibit unusual aqueous solution behavior, arising from hydrophobic assocations that occur in order to minimize water–hydrophobe contact.[2-6] Such associations determine the macromolecular conformation, which, in turn, controls the rheological properties of aqueous fluids. These polymers have potential applications in such diverse fields as enhanced oil recovery, fluid modification, controlled drug release, personal care formulation, and frictional drag reduction.[7,8]

Certain hydrophobically modified polymer systems exhibit intermolecular association in aqueous solution[2,3] while others show a preference for intramolecular association.[1,4] The number of hydrophobic groups incorporated within the amphipathic polymers has been suggested to play an important role in determining the polymer conformation adopted in aqueous solution. Several polymer systems containing either hydrocarbon[9] or fluorocarbon[10] pendent chains show viscosity maxima with increasing hydrophobic group content, implying a transition from intermolecular to intramolecular association. Recent studies in our laboratory have indicated that the distribution of the hydrophobic groups along the polymer chain also significantly affects the conformation of such copolymers.[11,12] When the hydrophobic groups in pyrene-labeled acrylamido copolymer systems are arranged in a blocklike fashion with long intervening hydrophilic sequences, the hydrophobic interactions promote intermolecular association. Polymers with a random distribution of the same units, on the other hand, exhibit intramolecular associative behavior.

Although many hydrophobically modified polymer systems have been reported, much less attention has been focused on the control of the type of association by altering the polymerization process. As well, structure–property relationships for amphipathic copolymers in aqueous solution are not well established. This paper describes

the synthesis and solution properties of copolymers of acrylamide and dimethyldodecyl(2-acrylamidoethyl)ammonium bromide. The blockiness of the hydrophobic sequences can be controlled utilizing mixed micelle polymerization. Introduction of cationic groups into the polymers increases water solubility and allows arrangement of hydrophobes in blocklike structures using simple solution polymerization techniques. The length of hydrophobic blocks can be controlled under selected conditions. For comparison, a copolymer of identical composition but random hydrophobe distribution was prepared by solution polymerization in *tert*-butyl alcohol. The effect of random hydrophobe incorporation versus block hydrophobe incorporation on solution behavior was investigated. Changes in the run number of blocks were also examined. These data are interpreted in terms of inter- versus intramolecular associations.

Experimental Section

Materials. Acrylamide was recrystallized twice from acetone prior to use. Water was deionized to a conductivity of 1×10^{-17} mho/cm. Other materials were used as received.

Monomer Synthesis. Scheme I illustrates the synthesis of the hydrophobically modified acrylamido monomer dimethyldodecyl(2-acrylamidoethyl)ammonium bromide. Into a 250-mL three-necked round-bottom flask were added methylene chloride (82 mL), *N,N*-dimethylethylenediamine (8.82 g, 0.100 mol), and 6 N sodium hydroxide (25 mL) was placed in an ice bath and agitated by a magnetic stirrer under a nitrogen atmosphere. When the temperature dropped below 5 °C, acryloyl chloride (9.36 g, 0.104 mol) in 20 mL of methylene chloride was added slowly from an addition funnel such that the temperature was maintained below 10 °C. The mixture was stirred for an additional 30 min after complete addition of acryloyl chloride. The organic layer was then separated, washed twice with water and once with concentrated NaCl solution, and dried over anhydrous sodium sulfate. The solvent was removed on a rotary evaporator to yield a slightly yellow oil (11.67 g, 80% yield). The crude product was purified by vacuum distillation in the presence of a small amount of phenothiazine as an inhibitor. A colorless oil was collected at 88–90 °C under vacuum of 0.5 mmHg. IR (KBr) 3284 (N–H), 1655 (C=O) cm⁻¹; ¹H NMR (CDCl₃) δ 2.23 (s, 6 H), 2.46 (t, 2 H), 3.42 (m, 2 H), 5.55–5.60 (m, 2H), 6.25–6.27

Scheme I. Synthesis of DAMAB Monomer
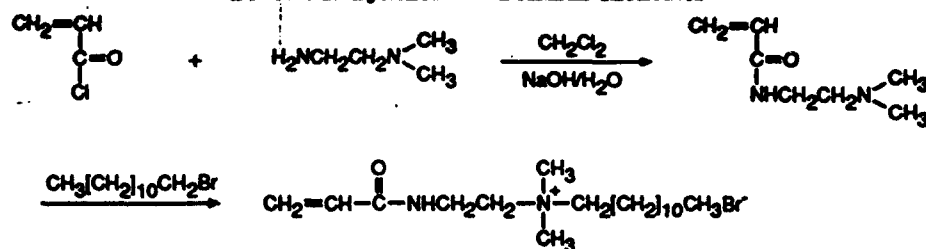
DAMAB

(m, 4 H), 7.53 (br, 1 H); ^{13}C NMR (CDCl$_3$) δ 36.45, 44.43, 57.29, 124.61, 130.59, 165.05.

Freshly distilled N-[(dimethylamino)ethyl]acrylamide (5.0 g, 0.035 mol) was then treated with dodecyl bromide (13 g, 0.052 mol) under nitrogen at room temperature for 48 h. Excess dodecyl bromide was decanted and the transparent gel precipitated into a white solid on cooling. The resulting material was washed with two portions of ether and dried under vacuum. Further purification of the final product was accomplished by recrystallization from a 1:1 mixture of acetone and ether. Yield 13.26 g (96%); mp 70.5–72 °C; ^{1}H NMR (CDCl$_3$) δ 0.88 (t, 3 H), 1.25–1.34 (br m, 20 H), 1.76 (m, 2 H), 3.42 (s, 6 H), 3.82 (m, 4 H), 5.63–5.74 (m, 1 H), 6.28–6.48 (m, 2 H), 8.79 (br, 1 H); ^{13}C NMR (CDCl$_3$) δ 13.18, 21.71, 21.97, 25.36, 28.36, 28.55, 28.65, 30.94, 33.18, 50.89, 51.18, 64.39, 165.56.

Copolymerization in the Absence of External Surfactant. To a 1000-mL three-necked round-bottom flask equipped with a mechanical stirrer, a condenser, and a nitrogen inlet were added acrylamide (AM) and dimethyldodecyl(2-acrylamidoethyl)ammonium bromide (DAMAB) in the desired ratio and 500 mL of deionized water. The total concentration of the comonomers was kept constant at 0.21 M. The solution was heated to 50 °C in a water bath with a small nitrogen stream passing through the system. Polymerization was then initiated by addition of K$_2$S$_2$O$_8$ (0.0262 g, 9.71 × 10^{-5} mol in 3 mL of deionized water) via a syringe. Polymerization was conducted continuously at 50 °C for 6 h, and then the reaction stopped by precipitating the polymer in 800 mL of acetone. The precipitated polymer was washed twice with acetone and vacuum-dried. Conversion was 60–72%. Further purification was accomplished by redissolving the polymer in water and dialyzing for a week against water using a 12000–14000 molecular weight cutoff dialysis tubing. The polymer was recovered by freeze-drying.

Copolymerization in the Presence of External Surfactant. Equimolar amounts of cetyltrimethylammonium bromide and DAMAB monomer were added with acrylamide to the polymerization system. The same procedure as in the previous case was followed for polymerization and polymer purification.

Solution Polymerization. A copolymer containing 5 mol % DAMAB in the feed was prepared via homogeneous solution polymerization. AM (23.3 g, 0.328 mol) and DAMAB (6.79 g, 0.0173 mol) were dissolved in 300 mL of tert-butyl alcohol. The solution was purged with nitrogen for 30 min at 50 °C. AIBN (0.056 g, 3.4 × 10^{-4} mol) was then added to initiate the polymerization. Polymerization was conducted for 10 h. In this case, the copolymer precipitated from solution as polymerization continued. The purification procedure was as described for the polymerization in water. A quantitative yield was obtained.

Characterization. ^{1}H and ^{13}C NMR spectra were recorded using a Bruker AC-300. A Mattson 2020 Galaxy Series FTIR was used to obtain infrared spectra. The critical micelle concentration of DAMAB was determined by surface tension measurements with a Kruss processor–tensiometer K12 instrument using the DeNouy ring method at 25 °C. Viscosity measurements were conducted with a Contraves LS-30 low-shear rheometer at a constant shear rate of 6 s^{-1} at 25 °C. Classical light scattering studies were performed on a Chromatix KMX-6 low-angle laser light scattering spectrophotometer with a 2-mW He–Ne laser operating at 633 nm. Refractive index increments (dn/dc) were obtained using a Chromatix KMX-16 differential refractometer. Steady-state fluorescence measurements were made with a Spex Fluorolog-2 fluorescence spectrometer. El-

Scheme II. Copolymerization of AM and DAMAB
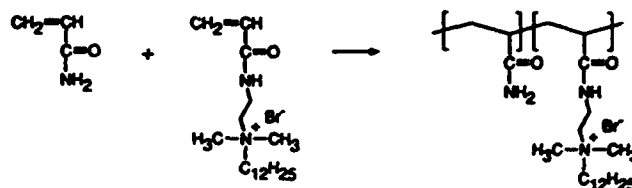
emental analyses to determine bromine content were conducted by MHW Laboratories of Phoenix, AZ.

Results and Discussion

Monomer Structure and Micellar Formation. In devising synthetic strategies for ionic monomers, it is generally better to generate the ionic structure in the last synthetic step to minimize any isolation and purification problems.[13] In our DAMAB monomer synthesis, the polymerizable group was first attached to the organic framework, followed by a single reaction which connected the hydrophobic group to the monomer and simultaneously generated the ionic structure. This two-step process proved to be very convenient, and the yield and purity of the product are quite satisfactory. The DAMAB monomer provides a quaternary ammonium group for enhanced solubility as well as the dodecyl group for hydrophobic association. The acrylamido functionality of the monomer provides a competitive copolymerization rate with acrylamide. Furthermore, this monomer has an amphipathic structure analogous to cationic surfactants; hence it will form micelles at concentrations above the critical micelle concentration (cmc). The cmc of this monomer was measured to be 4.9 × 10^{-3} M. Negligible change in the cmc was observed on addition of 0.21 M acrylamide. Kinetic studies have demonstrated that monomers of this type possess much higher polymerization rates due to aggregation of the monomers in water.[12] In our case, formation of micelles provides a high concentration of the reactive acrylamido groups near the micelle–water interface, facilitating the formation of the blocklike structures during the copolymerization with acrylamide.

Copolymer Synthesis. DAMAB and AM monomers were copolymerized successfully in water and in the presence of external surfactant (Scheme II). Incorporation of cetyltrimethylammonium bromide as the cationic cosurfactant should dilute the number of DAMAB molecules in each micelle; therefore, the blocks or "runs" of hydrophobic monomer units in the copolymer are expected to be shortened.

The solution polymerization was designed to yield a random copolymer. Copolymerization studies of AM (M$_1$) and DAMAB (M$_2$) in tert-butyl alcohol indicate that two monomers tend to undergo random, nearly ideal, copolymerization with r_1 = 1.14 ± 0.06 and r_2 = 0.88 ± 0.04. The degree of DAMAB incorporation in each of the copolymers was determined by elemental analysis for

Table I. Structural Parameters of the Copolymers

copolymer	DAMAB content (mol %)	$M_w \times 10^{-6}$
PAM	0	1.12[a]
R-C12-1	0.32	0.74[a]
BS-C12-4.3	4.3	0.95[b]
B-C12-4.7	4.7	1.04[b]
R-C12-5.1	5.1	0.46[b]
B-C12-10.5	10.5	1.12[b]

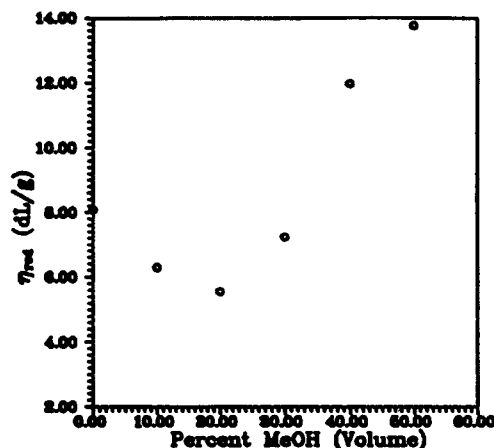[a] Measurement was taken in deionized water. [b] Measurement was taken in MeOH/H₂O mixture (50/50 by volume).

Figure 1. Effect of solvent on the η_{red} of R-C12-5.1 copolymer. The copolymer concentration is 0.127 g/dL.

Figure 2. Effect of the shear rate on the viscosity of R-C12-5.1 at 0.484 g/dL.

bromine content. The resulting compositions are summarized in Table I.

The copolymers are named according to their microstructures. The random and microblocky copolymers are distinguished by having R for "random" and B for "blocky" at the beginning of their names. C12 indicates that hydrophobes involved in the copolymers are dodecyl groups. The concentration of the hydrophobic groups in the copolymers are identified by the last number. For instance, R-C12-5.1 is a random copolymer containing 5.1 mol % dodecyl groups, and SB-C12-4.3 is a microblocky copolymer which was prepared in the presence of external surfactant and contains 4.3 mol % dodecyl groups.

Light Scattering Studies. For hydrophobically associating copolymers (BS-C12-4.3, B-C12-4.7, R-C12-5.1, and B-C12-10.5), methanol was used as a cosolvent in the light scattering measurement to disrupt hydrophobic associations and to keep the copolymers from interacting with the filter. As shown in Figure 1, for example, R-C12-5.1 in the methanol/water mixture (50/50 by volume) shows a higher viscosity than in deionized water. This is believed to be a result of breakage of intramolecular hydrophobic associations. Lack of hydrophobic associations for the copolymers in the mixed solvent is further demonstrated by studying the response of viscosity of the copolymer solution to changes in shear rate. As shown in Figure 2, a region in which the viscosity of R-C12-5.1 in water increases with increasing shear rate is observed. Below and above this region, the copolymer exhibits Newtonian flow. This shear thickening behavior has been reported by other researchers[3] and can be explained by changes in intra- and intermolecular associations. The intramolecular associations are disrupted above a certain shear stress, and chain extension results in an increase in the number of intermolecular associations. The viscosity of the same polymer in the methanol/water mixture only increases slightly with increasing shear rate, indicating a much less intramolecular hydrophobic association.

The weight-average molecular weight data for the copolymers are presented in Table I. The copolymer
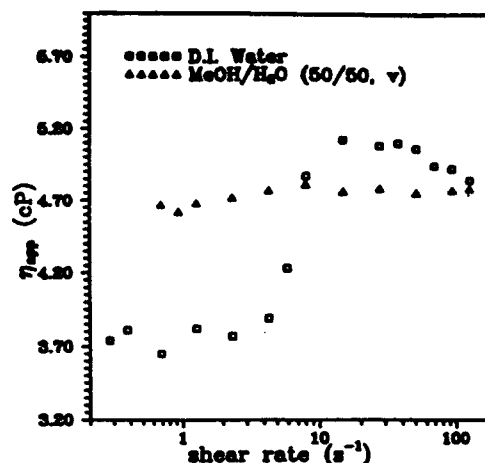
synthesized in *tert*-butyl alcohol has a substantially lower molecular weight than those prepared in water. However, the difference in the molecular weight for the two types of copolymers should not be taken alone as the reason for their different association behaviors.

Copolymer Solubility. Copolymers formed in the absence and presence of the cationic cosurfactant exhibit different solubilities. DAMAB monomer concentrations of 1, 5, and 20 mol % in the feed, respectively, result in complete water solubility. The copolymer with 25 mol % DAMAB monomer is only partially soluble, probably because of multiple associations. The effect is more pronounced for the copolymer containing a higher level of hydrophobe. Increasing DAMAB monomer concentration to 40 mol % results in total insolubility.

Dilute Solution Properties. Viscometry is a convenient and reliable method for determining associative properties of amphipathic copolymers in aqueous solution. A typical intramolecular associating polymer in aqueous solution is characterized by a lower viscosity compared to its parent polymer containing no hydrophobic groups. The viscosity will increase gradually with polymer concentration due to an increase in the hydrodynamic volume occupied by the macromolecule. If a rapid increase in apparent viscosity occurs at a critical concentration, C^*, the polymer is then described as intermolecularly associative in nature.

Associating properties of the copolymers can also be investigated by fluorescence spectroscopy using pyrene as a probe. The ratio of the pyrene fluorescence intensities of band I to band III (I_1/I_3) serves as an indicator of the polarity of the microenvironment. A lower value of I_1/I_3 indicates a more hydrophobic environment as experienced by the probe.[14]

Effect of Copolymer Composition. Before discussing the viscosity properties of the copolymers as a function of the copolymer composition, it is necessary to emphasize that since the copolymers are made at relatively high conversions, they are expected to have somewhat broad molecular weight distributions and copolymer compositions. Studies in Candau's group have shown that in the micellar copolymerizations both the molecular weight and hydrophobic content in the copolymers decrease as copolymerization runs to higher conversion.[15] In our experiments, the copolymerizations were terminated at 60–72% conversion and the DAMAB concentration remains above its cmc even at the highest conversion. Therefore, the number of the hydrophobic blocks in the copolymers may decrease as the copolymerization pro-
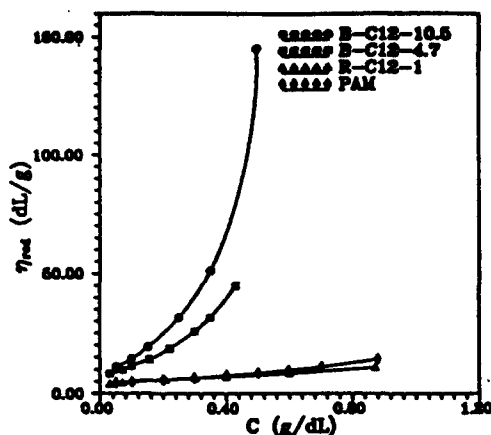
Figure 3. Effect of concentration on η_{red} for copolymers with various hydrophobic group contents in deionized water.
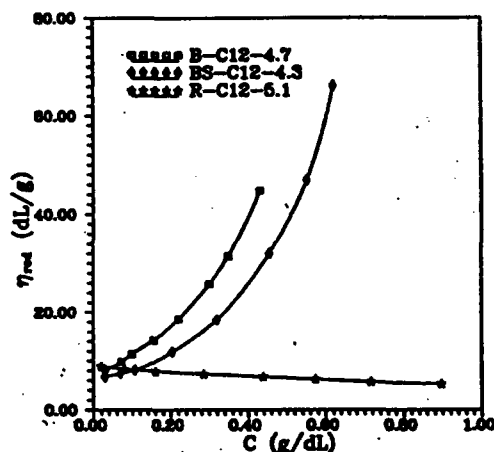

Figure 4. Effect of polymer concentration on η_{red} for copolymers containing various hydrophobic sequence lengths in deionized water.

ceeds, but the sequence length of the blocks should remain relatively constant.

Figure 3 illustrates the effect of polymer composition on solution properties. Viscosity increases with increasing DAMAB content. The copolymer containing 0.32 mol % of DAMAB (R-C12-1) displays viscosity behavior similar to that of polyacrylamide prepared under the same conditions; there are no significant hydrophobic associations in the concentration range investigated. Copolymers with higher DAMAB content show a significantly greater dependence of the viscosity on concentration, similar to the previously reported behavior of AM and *n*-alkylacrylamide copolymer systems.[2] The copolymer with 10.5 mol % DAMAB (B-C12-10.5) possesses a lower C^* and a steeper slope beyond C^* than the copolymer with 4.7 mol % DAMAB (B-C12-4.7). This is attributed to stronger intermolecular associations in the former. The C^* values of AM–DAMAB copolymers are generally higher than that of an AM and dodecylacrylamide copolymer containing 0.25 mol % dodecyl groups. This may be attributed to the presence of charged groups in the polymer chain. It is noteworthy, however, that no polyelectrolyte effect is observed for these systems in the dilute regime due to the low DAMAB concentration.

Addition of a cationic surfactant to the polymerization system containing 5 mol % DAMAB results in a lower slope gradient in the viscosity profile of the resulting polymer (BS-C12-4.3) relative to the one without external surfactant (B-C12-4.7) (Figure 4). This is consistent with formation of shorter blocks and/or a more random
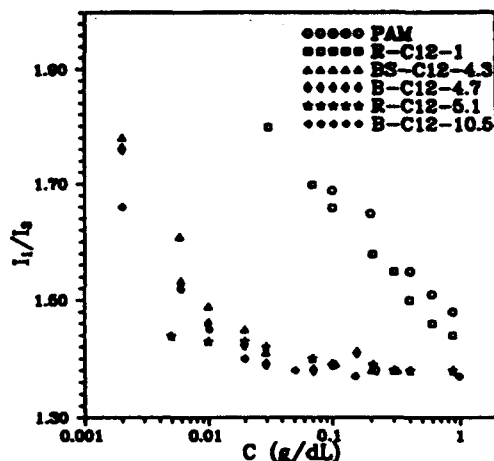

Figure 5. I_1/I_3 as a function of polymer concentration for various copolymers in deionized water.

distribution of DAMAB units, decreasing the tendency of intermolecular association.

A completely different viscosity profile for the copolymer with 5.1 mol % DAMAB prepared by solution polymerization (R-C12-5.1) is observed (Figure 4). The reduced viscosity decreases with increasing polymer concentration, indicating that hydrophobic associations are largely intramolecular in nature. This point will be addressed further in the discussion of fluorescence data and solvent effects.

Fluorescence Studies. Figure 5 depicts the dependence of I_1/I_3 values of pyrene steady-state fluorescence spectra on polymer concentration in aqueous solutions of the copolymers. I_1/I_3 values for BS-C12-4.3, B-C12-4.7, and B-C12-10.5 copolymers remain almost constant at 1.36–1.38 in the concentrations above 0.05 g/dL upon dilution. Further decreases in the copolymer concentrations result in rapid increases in I_1/I_3 values. The data suggest that pyrene molecules initially reside inside the hydrophobic microdomains formed through association of the hydrophobic groups. These hydrophobic microdomains vanish as polymer molecules are diluted to concentrations below 0.05 g/dL such that pyrene probes are exposed to a more aqueous environment. This behavior is consistent with intermolecular hydrophobic association. Furthermore, increase in I_1/I_3 occurs at concentrations well below C^*. This implies that hydrophobic interactions at the microscopic level take place at much lower concentration than reflected by macroscopic properties.

Pyrene fluorescence spectra for the R-C12-5.1 copolymer are consistent with classical water-soluble polymeric surfactant behavior[16] (Figure 5). A low I_1/I_3 value of pyrene throughout the copolymer concentration range is observed. This indicates that the R-C12-5.1 copolymer provides hydrophobic microdomains and acts as a host. The presence of hydrophobic microdomains is independent of copolymer concentration, consistent with intramolecular association.
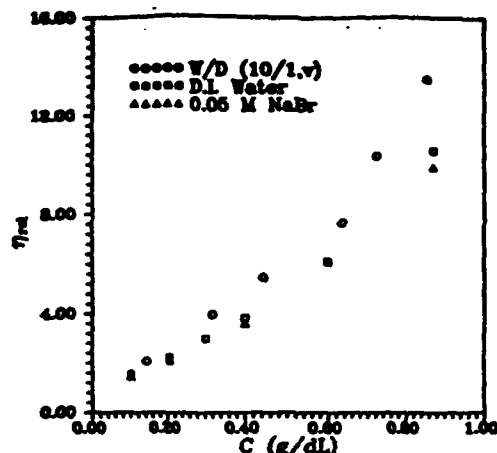
I_1/I_3 values in the R-C12-1 copolymer solution decrease continuously with increasing polymer concentration (Figure 5). A similar behavior is observed in polyacrylamide solutions. There is no well-defined transition point in the I_1/I_3 vs copolymer concentration curve, suggesting the absence of hydrophobic interactions between long alkyl chains capable of sequestering a pyrene probe from the aqueous environment. This information agrees favorably with that obtained from viscosity studies.

Effect of Solvent. Hydrophobic associations are induced by the water-structuring effect. Change in the
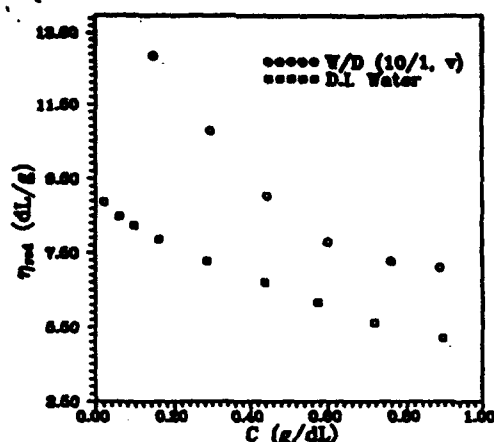
Figure 6. Effect of solvent on η_{red} of R-C12-5.1 copolymer.
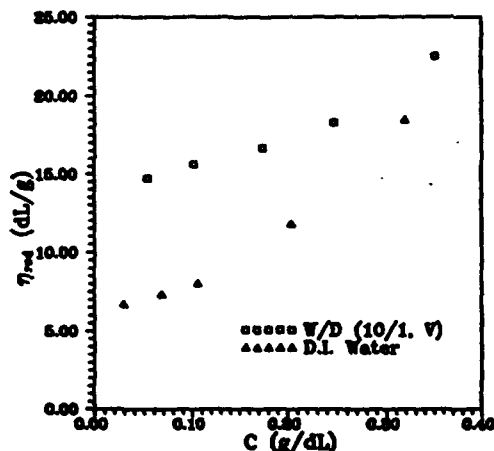
Figure 7. Effect of solvent on η_{red} of BS-C12-4.3 copolymer.

Figure 8. Effect of solvent on η_{red} of R-C12-1 copolymer.

water structure by addition of additives or cosolvents can either enhance or reduce hydrophobic interactions, depending on the nature of the additive. Controlled studies of changes in the rheological properties of polymer solutions in different aqueous media provide information on polymer conformation. In this work, NaBr is used as a water-structure-forming agent[17] and 1,4-dioxane as a water-structure-breaking agent.[18] The R-C12-1 copolymer is soluble in both 0.05 M NaBr aqueous solution and a water/dioxane mixture in a ratio of 10 to 1 by volume. However, solubility of the copolymers with higher hydrophobe content is affected significantly by added external electrolyte. Addition of NaBr results in precipitation from solution due to a "salting out" effect.[17] The copolymers are soluble in the water/dioxane mixture.

Figure 6 shows the effect of solvent on the viscosity of the R-C12-5.1 copolymer. The reduced viscosity increases upon addition of 10% dioxane. This is attributed to the decrease in the extent of intramolecular association resulting in the expansion of the polymer coil. Intermolecular association is also disrupted as dioxane is introduced into BS-C12-4.3 and B-C12-4.7 copolymer solutions. The viscosity of the BS-C12-4.3 copolymer shows less concentration dependence due to reduced intermolecular association tendency (Figure 7). It is interesting to note that this copolymer possesses a higher reduced viscosity in the lower concentration range in the water/dioxane mixture than in deionized water. A similar behavior is also observed for the B-C12-4.7 copolymer. A possible explanation is that there are some intramolecular associations of hydrophobic groups in the low-concentration regime which are disrupted upon addition of dioxane. This indicates that hydrophobic associations undergo a tran-

sition from intermolecular to intramolecular when the solution is diluted. A similar observation was reported by Siano et al.[19] in a study of acrylamide and n-octylacrylamide copolymers using 8-anilino-1-naphthalenesulfonic acid as a fluorescence probe. Schulz et al.[20] also observed such a transition for the copolymer of acrylamide and a nonionic surfactant monomer. However, such an intramolecular association at low concentration was not seen in pyrene probe studies. This suggests that while polymer solution properties may be influenced considerably by the presence of hydrophobic interactions, aggregates may not be of sufficient size to protect pyrene molecules from the bulk aqueous solution. I_1/I_3 values of pyrene fluorescence in such cases fail to "report" the presence of hydrophobic interactions.[21] The viscosity profile of the R-C12-1 copolymer is essentially invariant with addition of NaBr or dioxane, again indicating the lack of hydrophobic interactions in this system (Figure 8).

Conclusions

Rheological and fluorescence studies of amphipathic copolymers of AM and DAMAB have demonstrated that the associating behavior of the copolymers is governed by the arrangement of DAMAB units along the polymer chains. A random copolymer with 5 mol % of DAMAB shows a tendency for intramolecular hydrophobic association, while a microblocky copolymer of identical composition results in intermolecular association of hydrophobes. The intermolecular hydrophobic associations are enhanced by increasing the length of the hydrophobic block and/or the number of blocks in the polymer chain.

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Water-Soluble Copolymers. 50. Effect of Surfactant Addition on the Solution Properties of Amphiphilic Copolymers of Acrylamide and Dimethyldodecyl(2-acrylamidoethyl)ammonium Bromide

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ABSTRACT: The interactions of surfactants sodium dodecyl sulfate (SDS), trimethyltetradecylammonium bromide (TTAB), and Triton X-100 with amphiphilic copolymers of acrylamide and dimethyldodecyl(2-acrylamidoethyl)ammonium bromide (DAMAB) have been investigated in aqueous solutions. The rheological properties of a copolymer/surfactant system are affected by both the microstructure of the copolymer and the nature of the surfactant. Addition of the nonionic surfactant, Triton X-100, resulted in a large increase in the reduced viscosity for the microblocky copolymers with 5 mol % DAMAB, while a random copolymer with the same composition exhibited a collapsed conformation in the presence of the cationic surfactant, TTAB. A strong viscosity enhancement was observed when SDS was added to the solution of a copolymer containing 0.32 mol % DAMAB. Evidence of mixed micelles formed by surfactant molecules and the hydrophobic groups of the copolymers was obtained utilizing surface tension, pyrene probe fluorescence, and viscometry.

Introduction

The solution properties of aqueous media can be dramatically changed by utilizing combinations of amphiphilic copolymers and simple surfactants.[1] However, there is presently an inadequate understanding of the mechanism of the interactions between macromolecules and surfactants.[2] Aqueous solutions comprised of polymers and surfactants have been studied by a variety of experimental techniques including viscometry, surface tension, dialysis equilibrium, NMR, ESR, neutron scattering, and fluorescence. In general, the surfactant molecules form micelle-like clusters associated with the polymer chains. The behavior of these systems can be described by two critical surfactant concentrations, namely, the critical aggregation concentration (C_1) and the apparent critical micelle concentration (C_2). C_1 is defined as the surfactant concentration required to induce surfactant binding to the polymer, and C_2 is the concentration at which all the polymer sites available for interaction with surfactant are saturated; further increase in surfactant concentration will lead to the formation of classical micelles.[3,4] C_1 and C_2 are always located, respectively, below and above the critical micelle concentration (cmc) of the corresponding surfactant in pure water solution. C_1 is largely independent of the polymer concentration, while the maximum amount of polymer-bound surfactant in the system increases linearly with the total polymer concentration in the solution.[5]

Interactions between polyelectrolytes and charged surfactants in aqueous solution are governed primarily by Coulombic forces and dipole–ion interactions. For example, polyelectrolytes interact strongly with oppositely charged surfactants which can lead either to a large increase in solution viscosity[6,7] or in some cases to phase separation.[8] Often the associations between polyelectrolytes and surfactants are quite weak or absent mainly because of the unfavorable electrostatic repulsions.[2b,9] When polyelectrolytes are hydrophobically modified, the situation becomes more complicated. In addition to the forces operating in simple polyelectrolyte/surfactant systems, hydrophobic interactions between the amphiphilic groups

on the polymer chains and the surfactant molecules also play important roles. For instance, a C_{12}-grafted hydroxyethyl cellulose[10] exhibits strong association with SDS while its parent polymer,[11] hydroxyethyl cellulose, interacts only weakly with SDS.

Another major consideration affecting amphiphilic polymer/surfactant interactions is the chemical microstructure of the polymer, particularly the "mer" distribution resulting from the method of polymerization.[12,19,20] Change in the compositional distribution can dramatically alter the association of surfactant with the polymer. Addition of SDS to an aqueous solution of hydrophobically modified poly(sodium acrylate) has been shown to cause a marked viscosity enhancement.[13] The viscosity passes through a pronounced maximum in the region of the critical micelle concentration. This viscosity increase is observed only when the polymer concentration is in the semidilute regime and it is attributed to mixed micelle liasons between the hydrophobic elements of the chains. The decrease in viscosity above the cmc arises from stoichiometry, since the mixed micelles eventually will contain, on average, only one polymer alkyl chain per micelle, and cross-linking will be lost because there is no longer any stoichiometric requirement for the polymer hydrophobes to share micelles. Similarly, studies for hydrophobically modified acrylamide copolymers in the presence of SDS suggest that the surfactant disrupts intermolecular association of the hydrophobic groups, causing a decrease in solution viscosity.[14,15] In viscometric studies, Goddard et al.[6] investigated the effect of the polymer structure on the interactions of cationic polymers and an anionic surfactant system. Their studies indicated that addition of SDS to a cationically modified cellulosic terpolymer leads to intermolecular association between polymer chains via bound surfactant at high polymer concentrations (1% by weight) but intramolecular association at low concentration (0.1%). A similar investigation of the interaction of SDS with poly(acrylamide–co–(β-methacryloxyethyl)trimethylammonium chloride) showed no detectable change in viscosity over the same SDS concentration range, and it was concluded that the latter polymer maintained a constant conformation during binding of the surfactant.[17,20] In an analogous study, Hayakawa et al.[16] investigated the
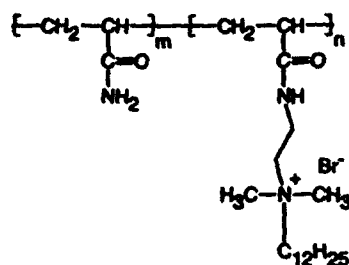
Figure 1. Structure of AM/DAMAB copolymers.

interaction of trimethyldodecylammonium bromide with poly(styrenesulfonate) and dextran sulfate. The equilibrium binding constant is higher for poly(styrenesulfonate) than for dextran sulfate, but, surprisingly, the cooperativity of the binding is higher for the more hydrophilic dextran sulfate. Thus higher hydrophobicity in a polymer does not necessarily result in enhanced micellar clustering of surfactant on the polymer; other factors such as linear charge density and flexibility of the polymer must also be taken into account.

In previous work, we described the synthesis and solution properties of copolymers of acrylamide and dimethyldodecyl(2-acrylamidoethyl)ammonium bromide (Figure 1).[17,20] These copolymers contain various amounts (up to 10 mol %) of dodecyl groups and show very interesting hydrophobic associative properties in aqueous solutions. Associative behavior of the copolymers is affected to a large degree by the arrangement of the hydrophobic groups along the copolymer chain. When the hydrophobic groups in such copolymers are arranged in a blocklike fashion, the hydrophobic interactions promote intermolecular association when the copolymer concentration is above C^*. A random copolymer with the same composition exhibits intramolecular associative behavior and reaches C^* at much higher concentration. The intermolecular interactions can be enhanced by increasing the length of the hydrophobic blocks and/or the number of blocks in the copolymer.

In this paper we report studies of the effects of sodium dodecyl sulfate, trimethyltetradecylammonium bromide, and Triton X-100 on the solution properties of copolymers of acrylamide with dimethyldodecyl(2-acrylamidoethyl)-ammonium bromide. Surfactant concentrations were chosen to span a range from below to above the cmc of the surfactants in pure water. Rheological behavior as a function of the type of surfactant and the distribution of the hydrophobic groups is investigated. Complementary data obtained by surface tension and pyrene probe fluorescence are also presented.

Experimental Section

Materials. Deionized water used in this study has a resistivity of 18 MΩ and a surface tension of 70.8 mN/m. All surfactants were purchased from Aldrich (purity: SDS, 98%; TTAB, 99%; Triton X-100, containing <3% poly(ethylene glycol)). The syntheses of the copolymers have been described elsewhere.[17,20] Microblocky copolymers were prepared by free-radical polymerization in aqueous solution with the surfactant monomer concentration above cmc (BS-C12-4.3, B-C12-4.7). Two random copolymers were synthesized via solution polymerization techniques: one in tert-butyl alcohol (R-C12-5.1) and the other in water with the surfactant monomer concentration below the cmc (R-C12-1). The compositions of the copolymers are summarized in Table 1. The DAMAB content was determined by elemental analysis, and the weight-average molecular weights of the copolymers were measured with a Chromatix KMX-6 low-angle laser light scattering spectrophotometer in methanol/water
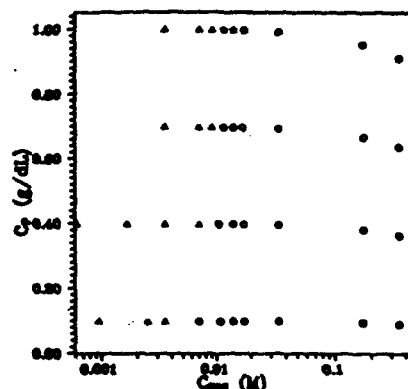

Figure 2. Solubility diagram of R–C12–1/SDS: two phases (Δ); clear solution (O); gel (☆).

Table 1. Composition of Copolymers

copolymer	DAMAB content (mol %)	$M_w \times 10^{-6}$
PAM	0	1.12
R–C12–1	0.32	0.74
BS–C12–4.3	4.3	0.95
B–C12–4.7	4.7	1.04
R–C12–5.1	5.1	0.47
B–C12–10.5	10.5	1.12

Methods. Surface tension measurements were performed with a Kruss Processor Tensiometer K12 instrument at 25 °C using a Pt DeNouy ring. Viscosity measurements were conducted on a Contraves LS-30 low-shear rheometer at 25 °C and a shear rate of 6 s⁻¹. Elemental analyses were conducted by MHW Laboratories of Phoenix, AZ. Steady-state fluorescence spectra were obtained with a Spex Fluorolog 2 fluorescence spectrophotometer equipped with a DM3000F data system. Classical light scattering studies were performed with a Chromatix KMX-6 low-angle laser light scattering spectrophotometer with a 2-mW He–Ne laser operating at 633 nm. Refractive index increments for classical studies were obtained on a Chromatix KMX-16 laser differential refractometer. A Spectra-Physics 127 laser operating at 632.8 nm was used for dynamic light scattering studies. Data were collected using a Brookhaven Instruments Model BI-2000SM automatic goniometer interfaced with a Brookhaven Instruments personal computer. Studies were performed at 90°, and the signals were processed with a Brookhaven Instruments Model BI-2030AT autocorrelator. Data were analyzed using the algorithm CONTIN and associated software provided by the manufacturer. Polymer solutions of 2.0 × 10⁻⁴ g/mL in 0.5 M NaCl were filtered in a Tygon tubing filter loop using Millipore 0.45-μm filters to remove dust. Typical filtration times were 14–48 h. Multiple analyses were performed to ensure reproducibility.

Results and Discussion

Anionic Surfactant. Solubility. The solubility diagram for the copolymer R–C12–1/SDS system in deionized water is shown in Figure 2. At low polymer concentrations (C_p <1%), the system exhibits several distinguishable solubility zones. When SDS is initially added to the copolymer solution, turbid two-phase dispersions are observed. Continued addition of the surfactant results in macroscopic phase separation in which one clear phase is on top of the other clear phase. The precipitate is resolubilized in the presence of excess surfactant, and the resulting solutions are clear and fluid. This solubility behavior is similar to that reported by Goddard et al.[18] Note that the transition occurs at the cmc of SDS. Initial precipitation of the copolymer in this case is caused by interaction of SDS with the copolymer by opposite ion charge interaction between surfactant and polymer ions, and this simultaneously increases hydrophobicity of the copolymer. At higher surfactant con-
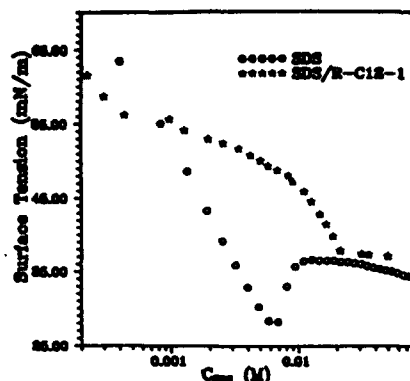
Figure 3. Surface tension measurements of SDS in the presence and absence of R–C12–1 copolymer (C_p = 0.33 g/dL).

Figure 4. η_{red} and I_1/I_3 as a function of SDS concentration for R–C12–1 copolymer at a concentration of 0.33 g/dL (C_{pyr} = 10^{-6} M).

centrations, however, it is likely that the SDS forms micelle-like clusters around hydrophobic groups attached to the polymer, and the copolymer is resolublized.

When copolymer concentrations are above 1%, a gel phase is observed at the surfactant concentrations between the precipitating zone and the resolubilizing zone. Apparently, liaisons result in a physical cross-linking network requiring association between SDS and the copolymer; no such gel formation is observed in the pure copolymer solutions.[19]

Phase separation occurs when SDS is mixed with B–C12–4.3, B–C12–4.7, and R–C12–5.1 copolymer solutions throughout the copolymer concentration range. Dubin et al.[20] have shown that the degree of ion pairing between high charge density polyelectrolytes and oppositely charged micelles is very extensive so that complexation leads to the rapid and irreversible formation of an amorphous solid, much as the mixing of oppositely charged strong polyelectrolytes produces water-insoluble "polyelectrolyte complexes".[21] In our study, precipitation of the copolymers upon addition of SDS may be caused by this strong ion-pair interaction. Because of solubility limits, studies of the interaction of SDS and copolymers were focused on SDS/R–C12–1 systems.

Surface Tension. Surface tension measurements show a classical "crossover" of the "with- and without-polymer" surface tension curves for the SDS solutions[22] (Figure 3). The surface tension of the SDS solution in the absence of the copolymer displays a pronounced minimum in the region of the cmc. This has been previously reported as a result of the presence of a lauryl alcohol impurity in the surfactant.[23]

The concentration, C_1, at which the surfactant begins to interact with the polymer and the concentration, C_2, at which the normal micelles begin to form are conspicuous in Figure 3. It is interesting to note that in the presence of the copolymer the surface tension curve shows no minimum. It can be inferred from the shape of the curve that lauryl alcohol is comicellized in the polymer–surfactant complex in preference to being adsorbed at the air–liquid interface. When regular micelles form, the measured surface tensions are slightly higher in the presence of copolymer. This may be an artifact of the DeNouy method, arising from viscoelastic recoil of the solubilized copolymer. The lower value of the copolymer curve at surfactant concentrations below C_1 can be explained by the surface activity of the copolymer. Adsorption of copolymer at the surface would necessarily compress the area available for surfactant adsorption, which would, in turn, increase the surface excess surfactant concentration and, consequently, cause a lowering of the surface tension. The higher surface tension at a surfactant
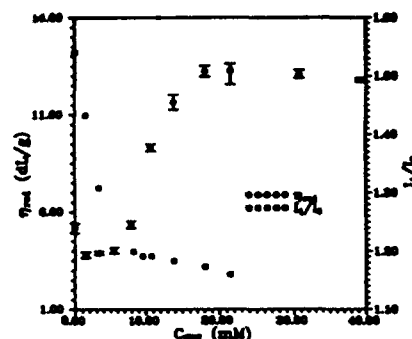
concentration around the cmc clearly demonstrates binding of the surfactant to the copolymer and concomitant depletion of surfactant from the air–water interface. C_1 in this case is 4.5×10^{-4} M. The surfactant adsorption on the polymer reaches a limit at a surfactant concentration of 9.7×10^{-3} M, corresponding to 60 SDS molecules per DAMAB monomer. This agrees well with the value of 50–60 surfactant molecules per micelle cluster reported in previous literature.[24]

Viscosity and Fluorescence Measurements. Figure 4 illustrates the dependence of the reduced viscosity of R–C12–1 copolymer on the SDS concentration. Initial addition of SDS causes a slight decrease in the viscosity, presumably due to the loss of the copolymer from the solution which could arise from mixed micelle formation within each single amphiphilic copolymer molecule in solution. On the other hand, I_1/I_3 decreases in the region until a constant value is reached at a surfactant concentration of 5.1×10^{-3} M, a concentration significantly lower than the cmc of SDS (8.3×10^{-3}). It is noteworthy that I_1/I_3 decreases gradually with increasing SDS concentration over a large surfactant concentration range, indicating that the binding of charged surfactant occurs by a noncooperative mechanism.[25] It is likely that surfactant molecules first adsorb on DAMAB monomer units at low surfactant concentrations to form hydrophobic clusters. Continuous addition of the surfactant results in an increase in cluster size; a hydrophobic microdomain is formed at a critical concentration of 5.1×10^{-3} M. The ratio of SDS molecules to DAMAB monomers in each microdomain is 34 as estimated from fluorescence data in Figure 4. This value is lower than that determined from surface tensiometry but is reasonable. It appears that not all of the polymer hydrophobes are comicellized at C_1. The transition point of the curve corresponds to the surfactant concentration at which the precipitate begins to resolubilize. This clearly implies that micelle type aggregates are responsible for redissolution of the precipitate.

At the cmc of SDS, further addition of surfactant dramatically enhances the solution viscosity. The curve reaches a plateau value at 18 mM SDS. This viscosity behavior differs from that observed by Leung and Goddard[6] for a cationic cellulose ether and SDS. In the latter case, the highest viscosity was observed in the precipitation zone, and viscosity dropped sharply in the resolubilization zone. In our system the increase in viscosity may be ascribed to association of surfactants and DAMAB of the copolymer in mixed hydrophobic microdomains. It is reasonable to assume that adjacent hydrophobic microdomains combine with increasing SDS concentration. Larger clusters then form which may contain DAMAB units belonging to two or more distinct polymer chains. Taking into account that the copolymer concentration
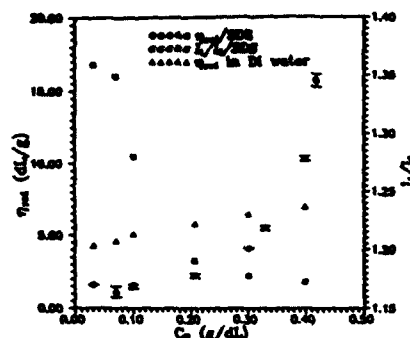
Figure 5. η_{red} and I_1/I_3 as a function of concentration of R–C12–1 copolymer in deionized water and 8.32 mM SDS aqueous solution.
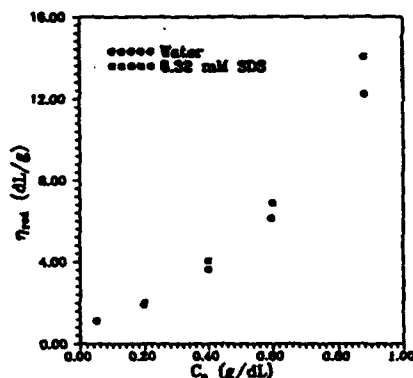

Figure 6. Effect of SDS on the η_{red} of homopolyacrylamide.

Figure 7. Surface tension measurements of Triton X-100 in the presence and absence of R–C12–1 copolymer (C_p = 0.33 g/dL).

Figure 8. η_{red} and I_1/I_3 as a function of Triton X-100 concentration for R–C12-1 copolymer at concentration of 0.33 g/DL (C_{pyr} = 10^{-6} M).

studied is in the semidilute regime, these microdomain clusters serve as sites where hydrophobic groups from adjacent polymer chains associate intermolecularly.

Formation of intermolecular association in this system can be seen more clearly by studying the viscosity behavior at fixed surfactant concentrations. As shown in Figure 5, a sharp upturn in the viscosity occurs within a narrow concentration range in the system containing 8.32 mM SDS, typical of intermolecular association. Such viscosity enhancement is not observed for polyacrylamide (Figure 6). Obviously, this interchain association is facilitated by interactions between SDS and DAMAB. It is interesting to note that the viscosity upturn occurs at the concentration where a constant limiting value of I_1/I_3 is reached. This value is very close to the one in Figure 4, suggesting that pyrene molecules locate in similar mixed micelles.

Clearly, two effects promote DAMAB association with SDS, hydrophobic and ionic associations. Hydrophobic associations reduce the contacts of the hydrophobic groups of the copolymer with water and consequently lower the free energy of the system.[26] Ionic attractions between SDS molecules and the cationic groups of the copolymer also contribute to the formation of mixed micelles. Each micelle apparently contains two or more DAMAB moieties belonging to different polymer chains.

Nonionic Surfactant. Surface Tension. It has been shown that, in a system composed of a nonionic surfactant and a polymer having long hydrocarbon pendent groups, surfactant molecules form micelle-like clusters adsorbed to each hydrocarbon chain.[2a,14,27,28] Such associations also occur when Triton X-100 is added to the R–C12–1 copolymer solution (Figure 7). C_1 is 0.032 mM and C_2 is 0.40 mM.

Viscosity and Fluorescence Studies. The effects of nonionic surfactant on the viscosity of C12–1 copolymer solution are shown in Figure 8. Contrary to behavior in SDS, addition of Triton X-100 to R–C12–1 solutions results in no observable change in the reduced viscosity. The
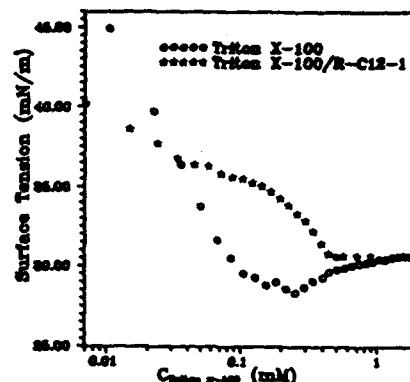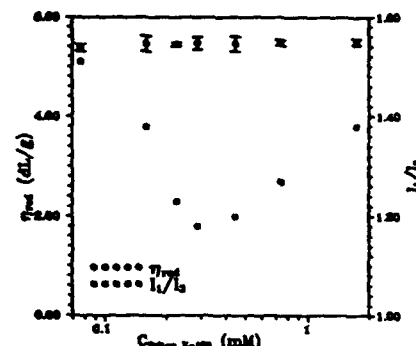
hydrodynamic size of the polymer coil remains nearly constant ($d_0 \approx 77$–79 nm) over a range of added surfactant concentration from 0.2 to 0.8 mM. However, I_1/I_3 decreases with increasing surfactant concentration, passing through a minimum and then increasing (Figure 8). The surfactant concentration at which I_1/I_3 shows the lowest value is very close to C_2. The initial decrease in I_1/I_3 is due to the formation of hydrophobic microdomains through association between surfactant molecules and hydrophobic groups of the copolymer. It is necessary to emphasize that the decrease in I_1/I_3 does not result from adsorption of surfactant to the pyrene molecules since the surfactant solution in the absence of the copolymer at the cmc shows a much higher I_1/I_3 of 1.44. When the limit of adsorption of the surfactant molecules onto the polymer is reached, further increase in surfactant concentration will result in the formation of normal surfactant micelles. Apparent I_1/I_3 values in such a system depend on the partitioning of pyrene molecules into two hydrophobic regions. By knowing the cmc of Triton X-100 in the system to be 0.3 mM and the C_1 of 3.2×10^{-2} mM, the number of surfactant molecules around each DAMAB unit is calculated to be ~3. Since there are no interactions between hydrophobes in the absence of surfactant, condensation of the surfactant onto the R–C12–1 polymer does not alter the polymer conformation, and the viscosity of the polymer solution is essentially invariant throughout the surfactant concentration range.

The effect of a nonionic surfactant on the viscosity of the polymer solution with higher hydrophobic content was investigated using the microblocky copolymer B–C12–4.3 (Figure 9). The polymer shows a higher viscosity in the presence of 0.264 mM Triton X-100 than in the absence of the surfactant. Apparently, this surfactant promotes intermolecular associations.

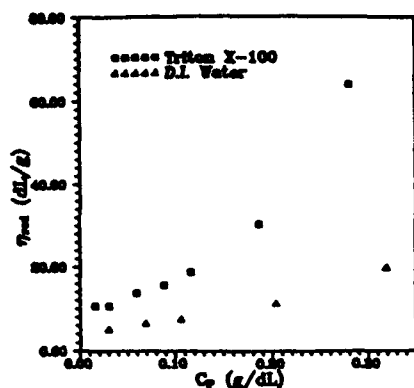**Cationic Surfactant. Surface Tension.** No definite

Figure 9. Viscosity behavior of B–C12–4.3 copolymer in 0.264 mM Triton X-100 aqueous solution and in deionized water.
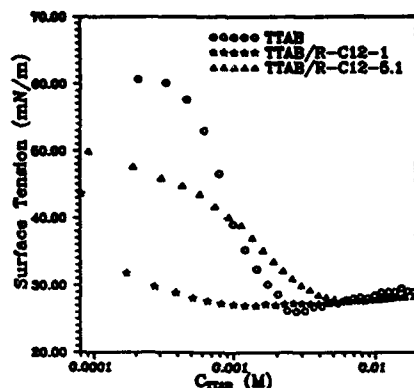

Figure 11. Effect of NaBr concentration on the surface tension of R–C12–1 and R–C12–5.1 solutions (C_p = 0.33 g/dL).

Figure 10. Surface tension behavior of TTAB in the presence of R–C12–1 and R–C12–5.1 copolymers (C_p = 0.33 g/dL).

Figure 12. η_{red} as a function of polymer concentration in the presence and absence of TTAB.

crossover is observed for the R–C12–1/trimethyltetradecylammonium bromide (TTAB) mixture (Figure 10). However, the system shows much more pronounced surface activity at very low surfactant concentrations, and the curve levels off below the cmc. Apparently, hydrophobic interaction overcomes ionic repulsion between the DAMAB moiety and the TTAB surfactant molecules, resulting in the formation of a surface-active complex. The polymer/surfactant association is also observed in the R–C12–5.1/TTAB system. In this case, however, the complex is less surface-active as compared with R–C12–1/TTAB. This may be explained by the collapsed conformation of R–C12–5.1 upon addition of TTAB as discussed below. The dramatic decrease in the surface tension in both R–C12–1 and R–C12–5.1 solutions around the cmc of the surfactant is not due to the exclusion of the copolymers from solutions to the air–liquid interface as a result of increased ionic strength. This is verified by only a slight decrease in surface tensions in both systems with increasing NaBr concentration (Figure 11). Addition of TTAB to microblocky copolymer solutions (B–C12–4.3, B–C12–4.7, and B–C12–10.5) results in polymer precipitation, probably due to a "salting-out" effect.[34]

Viscosity and Fluorescence Studies. Addition of TTAB does not change the rheological properties of the R–C12–1 copolymer. However, a significant decrease in viscosity is observed when TTAB is mixed with the R–C12–5.1 copolymer (Figure 12). In this case, TTAB acts as a cosurfactant associating with the polymer coils which become much more compact. The hydrophobic effect is apparently too strong in this system for the polymer coil to expand; the reduced viscosity remains essentially unchanged upon dilution. In other words, the copolymer behaves like a highly collapsed coil.
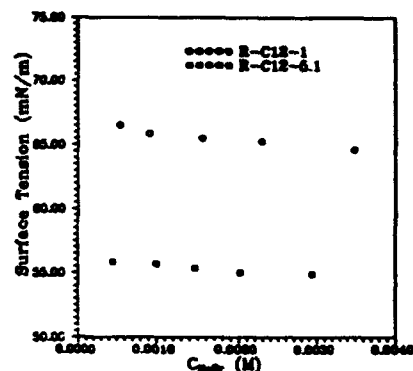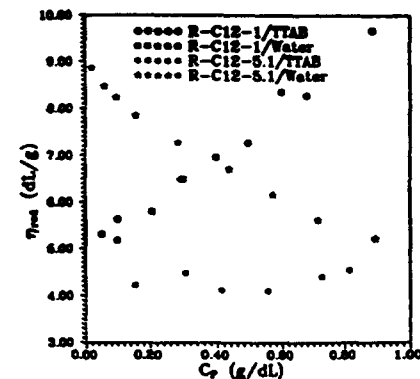
Conclusions

Effects of small surfactants on the rheological properties of the copolymers depend on the type of surfactant and the nature of the hydrophobic association (intermolecular vs intramolecular). Addition of SDS results in a slight decrease in the solution viscosity of R–C12–1 at surfactant concentration below the cmc and then a rapid increase at the cmc. The curve reaches a plateau at 18 mM SDS. This viscosity behavior can be explained by the association of SDS around each hydrophobic group along the polymer chain to form mixed micelles and subsequent polymer chain extension due to the ionic repulsion between the mixed micelles. The copolymers with higher hydrophobic content precipitate from the solution in the presence of SDS. In contrast, no significant viscosity change has been observed when the nonionic surfactant is added to a solution of R–C12–1 although the surface tension measurements and fluorescence studies indicate that association of surfactant with the copolymer does occur. An initial decrease and then an increase in the I_1/I_3 value can be explained by the partitioning of pyrene molecules between the homomicelles of Triton X-100 and the mixed micelles formed by hydrophobic groups of the copolymer and Triton X-100. Addition of nonionic surfactant to the SB–C12–4.3 solutions results in a large increase in the viscosity. In the case of TTAB cationic surfactant, R–C12–5.1 exhibits a collapsed conformation while the viscosity of R–C12–1 is hardly affected. However, R–C12–1 forms a more surface-active complex with CTAB than does R–C12–5.1. Macrophase separation occurs for the microblocky copolymer solutions in the presence of this cationic surfactant.

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Water-Soluble Copolymers. 58. Associative Interactions and Photophysical Behavior of Amphiphilic Terpolymers Prepared by Modification of Maleic Anhydride/Ethyl Vinyl Ether Copolymers

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ABSTRACT: A series of amphiphilic terpolymers were prepared by sequentially copolymerizing maleic anhydride and ethyl vinyl ether, adding octyl-, dodecyl-, or naphthylamine, and then hydrolyzing the remaining anhydride units. Viscosity studies indicate that these amphiphilic systems form intramolecular associations in dilute and semidilute solutions at various values of pH and ionic strength. The dodecyl terpolymers show more pronounced hydrophobic associations than do the octyl analogs. Change in solution pH or addition of NaCl lowers significantly the viscosity of the polymer solutions, indicating more collapsed conformations. Fluorescence lifetimes increase with increasing hydrophobe content. The lifetime of the monomer emission decreases with increasing pH, demonstrating a more hydrated environment experienced by the probe within the polymer coil. I_E/I_M values of the terpolymers with low hydrophobe content decrease with increasing pH from 3 to 10 while the terpolymers with moderate hydrophobe content exhibit a maximum I_E/I_M in the same pH range. Systems with high hydrophobe content show little pH dependence. These results are interpreted in terms of the relative concentration of the chromophore in the hydrophobic domains as well as the change in the mobility of the naphthyl labels.

Introduction

Intramolecular associative polymers, also called polymeric micelles or "polysoaps" due to their surfactant properties, were first studied as models to mimic the conformational behavior of proteins. Strauss and co-workers[1,2] demonstrated the formation of intramolecular micelles using the random copolymers of N-ethyl-4-vinylpyridinium bromide and N-dodecyl-4-vinylpyridinium bromide. The intrinsic viscosity decreased dramatically with increasing dodecyl group content and reached a minimum value of 0.04 dL/g at a hydrophobe content of 28.5 mol %. This value is of the same order of magnitude as that observed for globular proteins, indicating a highly collapsed conformation. The transition from extended to compact coils was reported to occur at a critical hydrophobe concentration of ~11 mol %. The Huggins constant exhibited an almost 25-fold increase as the dodecyl group content was changed from 0 to 37 mol %, verifying increased compaction of the polymer coils in aqueous media.

Loucheux et al.[3] studied the solution properties of poly-(4-vinylpyridine) modified with alkyl bromides utilizing viscometry and NMR techniques. Their data showed that a conformational transition occured only when the alkyl groups contained more than eight carbon atoms. The critical hydrophobe concentration shifted to lower value with increasing alkyl group length.

Another widely studied associative polymer is based on hydrolyzed alternating copolymers of maleic anhydride and n-alkyl vinyl ethers. These copolymers with moderate concentration of alkyl side chains (alkyl = butyl, pentyl, hexyl, octyl) in aqueous solutions undergo conformational transitions from hypercoil to random coil as the degree of ionization is increased.[4-12] The transition, however, is shifted to a higher value of ionization with increasing alkyl group size.[7] The conformation of the copolymers with longer hydrophobic chains (alkyl = decyl, cetyl) is essentially independent of the degree of ionization since these copolymers remain in a collapsed conformation at all values of pH.[13-15]

A series of studies have been conducted in our laboratories to further investigate the nature of intramolecular associations of various amphiphilic water-soluble polymers. Clark et al.[16] demonstrated the pH dependence of the chain associations in a 20:80 mol % copolymer of 2-(1-naphthylacetyl)ethyl acrylate (NAEA) and methacrylic acid (MAA). The copolymer adopts a highly collapsed conformation at low pH due to the hydrophobic interactions of both methyl and naphthyl groups. At high pH, the copolymer remains in a micelle-like conformation with the naphthyl groups forming a hydrophobic core surrounded by a charged shell of methacrylic acid units. Such a conformation is attributed to the presence of a large quantity of the naphthyl groups in the copolymer and the relatively long spacer between the naphthyl groups and the polymer backbone.

In this paper, we report the synthesis and solution properties of copolymers of maleic anhydride and ethyl vinyl ether reacted with controlled amounts of octyl- and dodecylamine. A small concentration (~1 mol %) of naphthalene labels with long spacer groups is incorporated. Hydrolysis of the remaining anhydride groups yields water-soluble terpolymers with degrees of amidation ranging from 8 to 50 mol %. Key features of this study include variation in hydrophobe length and content, maintenance of a small quantity of naphthyl groups to avoid perturbation of the parent copolymers, and the presence of comparatively large hydrophobes relative to the naphthyl label. These terpolymers provide useful models for elucidating the nature of hydrophobic associations in systems having a random distribution of the hydrophobic groups. Changes in polymer conformation upon altering the concentration and length of the hydrophobic groups as well as the solution pH and ionic strength are examined by viscosity and fluorescence studies.

Experimental Section

Materials. Chemicals were obtained from Aldrich Chemical Co. and used as received. Benzene was dried over CaH₂ and

**Scheme 1. Synthesis of
1-(((7-Aminoheptyl)oxy)methyl)naphthalene (3)**

**Scheme 2. Synthesis of Naphthalene-Containing Model
Compound 4**
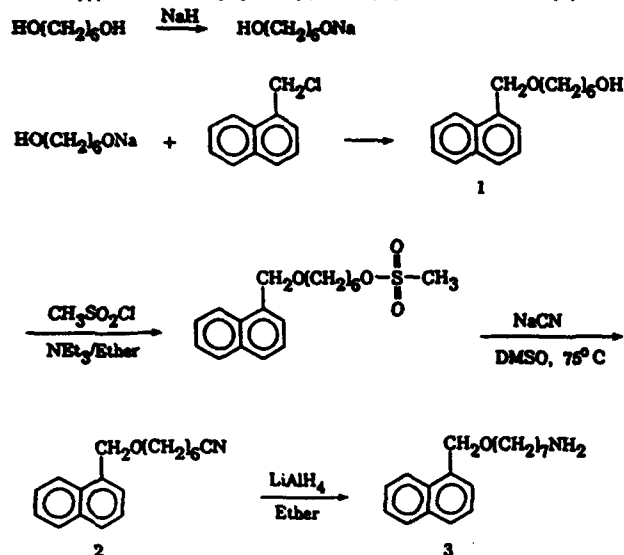
distilled under nitrogen prior to use. Other solvents were reagent grade and used without further purification. Water was deionized to a conductance of 1×10^{-17} mho/cm.

Synthesis of Naphthalene-Containing Fluorescence Label 3 (Scheme 1). 1-(((6-Hydroxyhexyl)oxy)methyl)naphthalene (1). 1,6-Hexanediol (100.0 g, 0.846 mol) was placed in a 1000-mL round-bottomed flask equipped with a mechanical stirrer and heated to melt at 55 °C. Sodium hydride (4.06 g, 0.169 mol) was added, and the mixture was stirred at 70 °C for 4 h until all NaH reacted. A precipitate was formed immediately upon addition of (chloromethyl)naphthalene (30.0 g, 0.169 mol). The mixture was stirred vigorously at 80 °C for 3 h. Thin-layer chromatography (TLC) indicated that most of the (chloromethyl)-naphthalene had been reacted. The reaction was allowed to proceed for another 3 h. Excess hexanediol was then removed at 106 °C/0.5 mmHg. The residue was diluted with 150 mL of acetone, and the precipitate was filtered. The concentrated filtrate was distilled under reduced pressure to yield a colorless oil: bp 166.5 °C at 0.5 mmHg, yield 41 g (94%). GC purity was determined to be >99.9%. ^{1}H NMR (CDCl$_3$) δ 1.22–1.70 (m, 8 H), 2.01 (s, 1 H), 3.47–3.55 (2 t, 2 H), 4.91 (s, 2 H), 7.39–8.15 (m, 7 H); ^{13}C NMR (CDCl$_3$) δ 25.5, 25.9, 29.7, 32.6, 62.6, 70.3, 71.4, 124.0, 125.1, 125.7, 126.0, 126.3, 128.5(2), 131.7, 133.7, 133.9; FTIR 3383 (O–H), 1597, 1510, 1460 (aromatic C–C stretch), 1099 (C–O–C), 794, 775 (aromatic C–H bend) cm^{-1}.

1-(((6-Cyanohexyl)oxy)methyl)naphthalene (2). A modified literature procedure[17] was used in the preparation of 2. Methanesulfonyl chloride (28.6 g, 0.250 mol) was added dropwise at 8–15 °C to a solution of 1-(((6-hydroxyhexyl)oxy)methyl)-naphthalene (51.7 g, 0.200 mol) and trimethylamine (30.4 g, 0.300 mol) in 150 mL of ether. After addition was complete, the mixture was stirred at room temperature for 1 h. The precipitate was filtered and washed twice with 50 mL of anhydrous ether. A light yellow oil was obtained from the filtrate upon removal of the solvent. ^{1}H NMR (CDCl$_3$) δ 1.29–1.73 (m, 8 H), 2.90 (s, 3 H), 3.52 (t, 2 H), 4.13 (t, 2 H), 4.91 (s, 2 H), 7.36–8.12 (m, 7 H); ^{13}C NMR δ 25.1, 25.6, 29.0, 29.5, 37.0, 70.1(2), 71.3, 124.0, 125.2, 125.7, 126.0, 126.3, 128.3(2), 131.7, 133.7, 134.0.

The oil was heated at 75 °C with sodium cyanide (15.5 g, 0.300 mol) in 200 mL of anhydrous DMSO for 3.5 h in a 500-mL round-bottomed flask. TLC confirmed the disappearance of the starting compound. The solution was cooled and poured into 800 mL of water. The product was extracted with 200 mL of methylene chloride three times. The organic extracts were combined and dried with MgSO$_4$. A dark red oil was obtained upon removal of the solvent. Vacuum distillation yielded a slightly yellow oil: bp 186–188 °C at 0.5 mmHg, yield 49.7 g (93%). ^{1}H NMR (CDCl$_3$) δ 1.20–1.66 (m, 8 H), 2.11 (t, 2 H), 3.46 (t, 2 H), 4.87 (s, 2 H), 7.32–8.11 (m, 7 H); ^{13}C NMR (CDCl$_3$) δ 16.8, 25.2, 25.3, 25.5, 28.2, 29.3, 69.9, 71.4, 124.0, 125.2, 125.7, 126.0, 126.3, 128.5(2), 131.7,
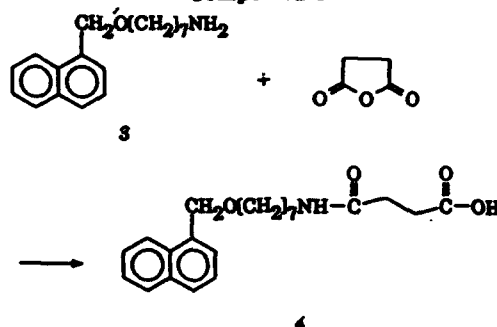
133.7, 133.9; FTIR 2245 (C≡N), 1597, 1510, 1462 (aromatic C–C stretch), 1099 (C–O–C), 801, 777 (aromatic C–H bend) cm^{-1}.

1-(((7-Aminoheptyl)oxy)methyl)naphthalene (3). LiAlH$_4$ (0.789 g, 0.0196 mol) in 40 mL of anhydrous ether was placed in a 100-mL flask equipped with a thermometer, an addition funnel, and a magnetic stirring bar. When the mixture was cooled to 0 °C, 1-(((6-cyanohexyl)oxy)methyl)naphthalene (5.00 g, 0.0187 mol) in 5 mL of ether was added slowly at such a rate that the temperature did not exceed 5 °C. After addition was complete, 1 mL of water was added slowly (*Caution*: very vigorous reaction), followed by 1 mL of 20% NaOH solution and an additional 3 mL of water. The ether layer was decanted from the white, granular inorganic residue. This residue was washed once with 20 mL of ether. The organic solution was dried with Na$_2$SO$_4$. Removal of ether yielded a slightly yellow oil. GC indicated that the product contained ~6% of 1-(((6-hydroxyhexyl)oxy)methyl)-naphthalene (1). To remove unreacted alcohol, the oil was dissolved in 10 mL of ether, and 20 mL of 1 M HCl ether solution was added. The ammonium salt was precipitated as an off-white solid which was filtered, washed with ether, and dried under vacuum. Recrystallization in acetone gave pure white crystals. The solid was dissolved in water, and the solution pH was adjusted to ~12. The free amine was extracted with ether. The organic phase was dried with Na$_2$SO$_4$, and the solvent was removed to yield a slightly yellow oil: yield 3.68 g (73%). HPLC purity was determined to be >99.8%. ^{1}H NMR (CDCl$_3$) δ 1.03 (b, 2 H), 1.19–1.70 (m, 10 H), 2.62 (t, 2 H), 3.52 (t, 2 H), 4.92 (s, 2 H) 7.34–8.16 (m, 7 H); ^{13}C NMR (CDCl$_3$) δ 22.9, 26.2, 26.8, 29.3, 29.7, 33.8, 42.3, 70.5, 71.4, 124.1, 125.1, 125.7, 126.0, 126.2, 128.5(2), 131.8, 133.7, 134.1; FTIR 3374, 3300 (N–H), 1597, 1512, 1464 (aromatic C–C stretch), 1101 (C–O–C), 792, 777 (aromatic C–H bend) cm^{-1}.

Succinic Acid N-[7-(1-Naphthylmethoxy)heptyl] Monoamide (4). The synthesis of naphthalene-labeled model compound 4 is illustrated in Scheme 2. 1-(((7-Aminoheptyl)oxy)-methyl)naphthalene (3) (1.00 g, 3.69 mmol) in 10 mL of anhydrous DMF was placed in a 25-mL flask equipped with a condenser, an addition funnel, and a N$_2$ inlet and outlet. Succinic anhydride (0.380 g, 3.80 mmol) in 5 mL of DMF was added at 0 °C with stirring. The reaction was conducted at room temperature for 30 min and at 65 °C for an additional 30 min. TLC confirmed disappearance of the amine. The product was precipitated in water. The light yellow solid was filtered, washed with 10 mL of water, and dried under vacuum: yield 1.19 g (87%). The product was recrystallized in ethyl acetate to yield a pale yellow solid: mp 78–79.5 °C. ^{1}H NMR (CDCl$_3$) δ 1.10–1.84 (m, 10 H), 2.39 (t, 2 H), 2.59 (d, 2 H), 3.09 (d, 2 H), 3.51 (t, 2 H), 4.90 (s, 2 H), 6.43 (b, 1 H), 7.31–8.19 (m, 7 H), 10.07 (b, 1 H); ^{13}C NMR (CDCl$_3$) δ 26.0, 26.7, 28.9, 29.1, 29.5, 30.0, 30.7, 39.7, 70.4, 71.3, 124.0, 125.2, 125.7, 126.1, 126.4, 128.5(2), 131.7, 133.7, 133.9, 172.6, 176.1; FTIR (KBr) 3460–2370 (O–H), 3319 (N–H), 1696 (acid C=O), 1643 (amide C=O), 1596 (aromatic C–C stretch), 1537 (amide II band), 1120 (C–O–C), 797, 767, (aromatic C–H bend) cm^{-1}.

Synthesis of the Sodium Salt of Hydrophobically Modified Maleic Anhydride and Ethyl Vinyl Ether Copolymers 7 (Scheme 3). Copolymerization of Maleic Anhydride (MA) with Ethyl Vinyl Ether (EVE). Maleic anhydride (10.77 g, 0.110 mol) and benzoyl peroxide (0.0451 g, 1.86 × 10^{-4} mol) were dissolved in 100 mL of benzene in a 250-mL three-neck round-

Scheme 3. Synthesis of Hydrophobically Modified Maleic Anhydride and Ethyl Vinyl Ether Based Terpolymers 7

R: $[CH_2]_7CH_3$, $[CH_2]_{11}CH_3$
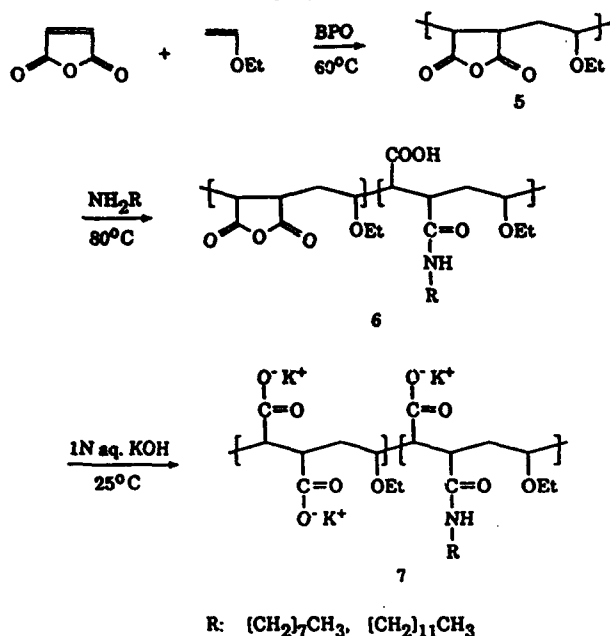
bottomed flask equipped with a magnetic stirring bar. The flask was sealed and fitted with a thermometer, a condenser, a long gas delivery needle, and a short needle on the top of the condenser to serve as a gas escape valve. Dry, oxygen-free nitrogen was purged through the flask for 45 min. The short needle was then removed and ethyl vinyl ether (9.11 g, 0.121 mol) was injected into the solution. The system was heated at 59–61 °C for 8 h, resulting in a white precipitate which was filtered and dried under vacuum. Copolymer 5 was purified by repeated precipitation in dry diethyl ether. The polymer was filtered and dried under vacuum.

Synthesis of Naphthalene-Labeled, Hydrophobically Modified Maleic Anhydride and Ethyl Vinyl Ether Terpolymers 6. Synthesis of naphthalene-labelled MA/EVE terpolymer containing 20 mol % of octyl groups is described below. Other terpolymers were prepared by the same procedure with differing amounts of alkylamines in the feed. 1-(((7-Aminoheptyl)oxy)methyl)naphthalene (0.162 g, 5.99×10^{-4} mol) (3) and octylamine (1.55 g, 1.20×10^{-2} mol) were added dropwise to a rapidly stirring solution of poly(maleic anhydride-*alt*-ethyl vinyl ether) (10.2 g, 5.99×10^{-2} mol) (5) in 100 mL of ethyl acetate at room temperature under nitrogen. The reaction was allowed to proceed at 60 °C for 10 h. The polymer was then precipitated into 300 mL of diethyl ether. The precipitate was stirred with 200 mL of diethyl ether overnight, filtered, and then dried under vacuum.

Hydrolysis of Hydrophobically Modified Maleic Anhydride and Ethyl Vinyl Ether Copolymers. The hydrolysis of the terpolymers 6 was performed in aqueous NaOH solution. A dilute NaOH solution containing an appropriate amount of dry polymer was placed on an orbital shaker at room temperature to facilitate the hydrolysis of the anhydride groups. After complete dissolution, the solution was dialyzed for 1 week against water at room temperature to remove low molecular weight oligomers and excess NaOH using dialysis tubing with a molecular weight cutoff of 12 000–14 000. The final products were obtained by freeze-drying.

Characterization. Naphthalene Derivatives. ^{1}H and ^{13}C NMR spectra were recorded using a Bruker AC-200. A Mattson 2020 Galaxy Series FTIR was used to obtain infrared spectra. GC analysis was performed on a Hewlett-Packard 5890 Series II gas chromatograph equipped with an Alltech AT-5 capillary column. A Hewlett-Packard Model 1050 HPLC was used to determine the purity of solid samples. A Waters Bondapak C18 column was employed with methanol as the mobile phase. The sample eluant was typically monitored at 280 nm.

Terpolymer Solution Preparation. The appropriate amount of dried terpolymer 7 was weighed and then dissolved in water in a volumetric flask from which further dilutions of this stock solution could be made. The solutions were allowed to stand for 2 weeks prior to viscosity measurement. The pH value of each solution was obtained with a Corning 130 pH meter at room temperature.

UV Analysis. Ultraviolet spectroscopy was used in determining the naphthalene content in the terpolymers. All spectra were recorded with a Hewlett-Packard 8452A diode array spectrophotometer. Beer–Lambert plots were obtained for the model compound, the sodium salt of succinic acid N-[7-(1-naphthylmethoxy)heptyl] monoamide (4) in water, and compared with polymer absorption.

Fluorescence Analysis. The concentrations of terpolymer solutions were 0.05 g/dL. The concentration of naphthyl moieties in these solutions varied from 2.5×10^{-6} to 3.0×10^{-4} M. Sample solutions were purged by bubbling with helium. All the samples were excited at 280 nm, and monomer intensities were measured at 330 nm. Emission spectra of the terpolymers were recorded with a Spex Fluorolog-2 fluorescence spectrometer. Fluorescence decays were measured with a Photochemical Research Associates (PRA) single-photon-counting instrument equipped with a H$_2$-filled 510-B flashlamp. A nonlinear iterative deconvolution technique was used to fit the decay curves.

Low-Angle Laser Light Scattering. Classical light scattering studies were performed with a Chromatix KMX-6 low-angle laser light scattering spectrophotometer with a 2-mW He–Ne laser operating at 633 nm. Refractive index increments (dn/dc) were obtained using a Chromatix KMX-16 differential refractometer. The molecular weight of hydrolyzed poly(maleic anhydride-*alt*-ethyl vinyl ether) was measured in 1 M NaCl solution.

Viscometry. Viscosity measurements were conducted with a Contraves LS-30 low-shear rheometer at a constant shear rate of 1.28 s^{-1} at 25 °C. A constant pH of 9–9.5 was used during the measurement, unless otherwise noted.

Results and Discussion

One synthetic objective of this work was to prepare amphiphilic water-soluble polymers with controlled placement of fluorescence labels. The random incorporation of the labels was achieved by first synthesizing the functionalized naphthalene derivative shown in Scheme 1. The synthesis of 1-(((7-aminoheptyl)oxy)methyl)-naphthalene (3) proved to be facile with satisfactory yields. The spacer length (in this case heptyl) can be altered to decouple the naphthalene from the polymer backbone. A model compound, succinic acid N-[7-(1-naphthylmethoxy)-heptyl]monoamide (4) (Scheme 2), was designed for fluorescence studies. The sodium salt form is soluble in aqueous media.

Terpolymer Synthesis and Characterization. Hydrophobically modified MA/EVE terpolymers 7 were prepared utilizing the synthetic procedures shown in Scheme 3. Terpolymers are designated by the number 7 followed by C8 or C12, designating octyl or dodecyl substitution. The final number represents the mole percent incorporation. Initially, MA/EVE copolymer 5 was prepared in benzene. Characteristic of this free-radical copolymerization is virtually complete alternation with little tendency of either monomer to homopolymerize.[18] The M_w of the copolymer obtained by light scattering in this study was 2.4×10^5 g/mol. The ^{13}C NMR spectrum of the MA/EVE copolymer is shown in Figure 1.

Initial attempts to induce the reaction of the MA/EVE copolymer 5 with alkylamines and naphthalene label 3 were performed in methyl ethyl ketone (MEK). The color of the products varied from pink to purple as the degree of incorporation of the hydrophobic groups increased from 8 to 50 mol %.
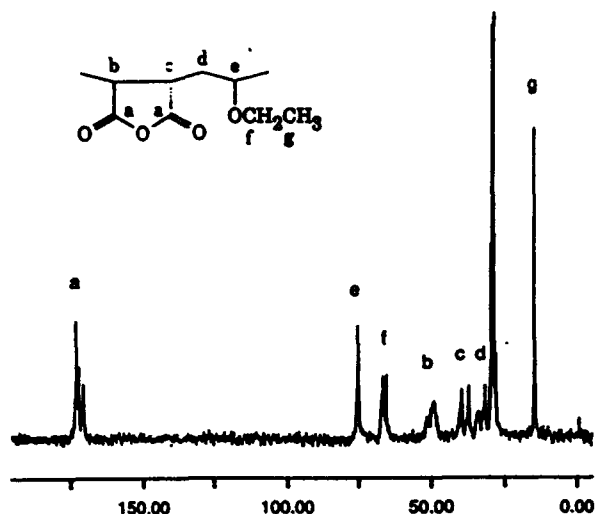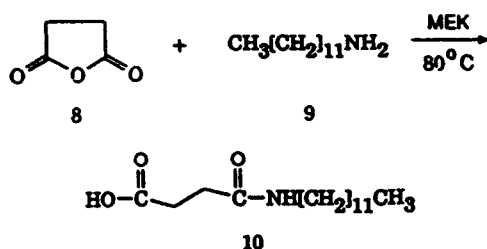
Figure 1. ^{13}C NMR spectrum of MA/EVE copolymer 5. Solvent: acetone-d_6.

Scheme 4. Model Reaction of Succinic Anhydride and Dodecylamine

8   **9**

10

To ascertain the possibility of charge-transfer complexation between the amine and anhydride functionality of the MA/EVE copolymer 5, we examined some model reactions. Reaction of succinic anhydride (SA) (8) with dodecylamine (9) yielded the corresponding amide 10 (Scheme 4) identified by NMR and FTIR. However, the solution of succinic anhydride and dimethyldodecylamine (DMDA) in MEK exhibited a color change from pink to brown at room temperature with increasing reagent concentration. It is known that pyridine can promote the hydrolysis of the anhydride group by forming a zwitterionic intermediate.[19,20] Kluger and Hunt[21] detected the formation of succinyl-4-(dimethylamino)pyridinium ion in the reaction of succinic anhydride with 4-(dimethylamino)-pyridine. It is likely that the color change in the succinic anhydride/dimethyldodecylamine solution is due to the formation of an analogous zwitterion. We also observed a similar color change for the reaction of the copolymer 5 with dimethyldodecylamine.

UV spectra of the solutions of SA/DMDA and the products from the reactions of the MA/EVE copolymer 5 with primary and tertiary amines exhibit the same absorption maximum at 556 nm (Figure 2), suggesting the presence of similar zwitterionic species in these three systems. The absorption intensity of the tertiary solution is stronger for the same feed ratio of amine to acid anhydride. This observation may be explained by partial dodecylamine conversion to the amide. This is consistent with the increase in intensities of the absorption at 562 nm for dodecylamine-modified terpolymers 7 as the degree of amidation varies from 8 to 50 mol % (Figure 3).

Reactions of the MA/EVE copolymer 5 with octyl- and dodecylamine in ethyl acetate exhibit no color changes, and ^{13}C NMR studies demonstrate that the products are the expected amide and carboxylic acid derivatives (Figure 4). In this solvent, homogeneous conditions should allow
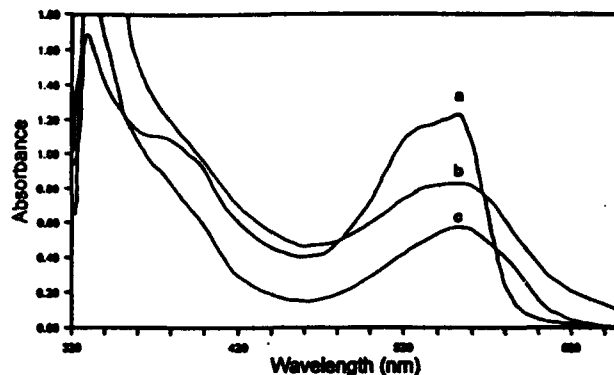

Figure 2. UV absorbance of 5/DMDA (a, 0.0036 g/mL), SA/DMDA (b, 0.036 g/mL), and 5/dodecylamine (c, 0.036 g/mL) obtained in MEK. Note that all three samples exhibit absorption maxima at 556 nm.
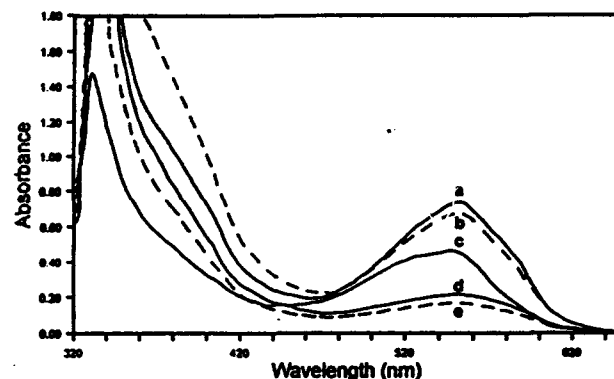

Figure 3. UV absorbance of 7-C12 terpolymers obtained in MEK. Sample concentration: 0.036 g/mL. (a) 7-C12-50; (b) 7-C12-40; (c) 7-C12-25; (d) 7-C12-16; (e) 7-C12-8.
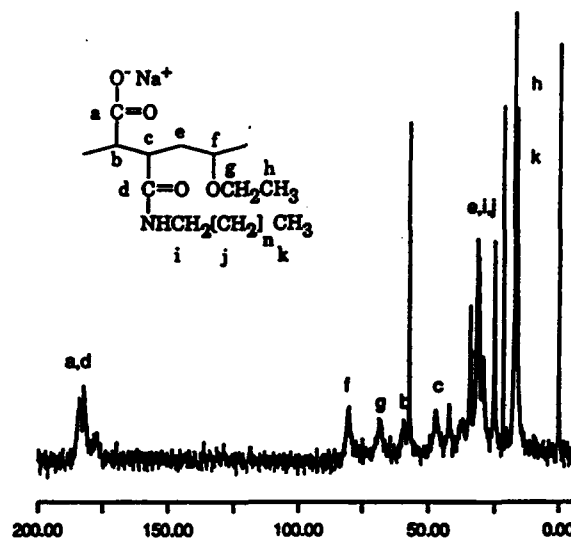

Figure 4. ^{13}C NMR spectrum of 7-C12-40 terpolymer. Solvent: D_2O.

uniform, random incorporation of the hydrophobic groups along the polymer chain. The hydrophobe concentration in the terpolymers can be easily controlled by the amount of the amine added to the reaction. To ensure complete conversion, the reactions were carried out at 80 °C for 10 h. It has been shown that 4-butylaniline reacts efficiently (100%) with the maleic anhydride and ethyl vinyl ether copolymer under the same reaction conditions.[22] Since the octyl- and dodecylamines used in this study are more reactive toward the anhydride group than aromatic amines, we feel confident that a complete reaction is also achieved. This is supported by the fact that the naphthalene content

Table 1. Terpolymer Compositions

terpolymer	hydrophobe content (mol %)	naphthalene content (mol %)
7-C8-10	10	1.05
7-C8-20	20	1.01
7-C8-30	30	0.995
7-C8-40	40	1.04
7-C8-50	50	1.04
7-C12-10	10	0.986
7-C12-20	20	1.00
7-C12-30	30	1.02
7-C12-40	40	1.06

in the terpolymers is the same as in the reaction mixture, about 1 mol %. The compositions of the terpolymers are summarized in Table 1.

The hydrolysis of the remaining anhydride groups of the modified terpolymers was performed in aqueous NaOH solutions at room temperature with the NaOH concentration varying from 0.1 to 0.5 M depending upon the degree of substitution. To completely dissolve the polymer with higher hydrophobe content, low NaOH concentration was necessary to avoid high ionic strength of the medium during neutralization. Complete hydrolysis was demonstrated through FTIR spectral analysis with disappearance of the C=O stretching vibration of the acid anhydride; it is assumed that no hydrolysis of the amide bond occurs since in previous work none was observed.[22] 7-C12-50 failed to dissolve completely in 0.01 M NaOH solution even over an extended period of time.

Solution Properties. The solution properties of amphiphilic water-soluble polymers are controlled by specific structural characteristics, molecular weight, and the solvent quality. The structural parameters including concentration and distribution of hydrophobic groups, hydrophilic segment length, and fixed ionic groups are among many which may be changed to yield the desired properties. For example, hydrophobic interactions of long-chain hydrocarbons incorporated along the polymer backbone can greatly affect the degree to which the polymer coil may expand or collapse. Changes in the degree of ionization and solvent characteristics such as ionic strength and pH alter the polymer conformations as well. In this study, influences of these parameters on the solution behavior of terpolymers 7 are examined by viscosity and fluorescence measurements.

Viscosity Studies. Aging Effects. Hydrophobically modified, water-soluble polymers often show time-dependent reorganization in solution.[23] To properly assess the effects of terpolymer composition, pH, and added electrolytes on solution behavior, aging studies were first conducted. Figure 5 shows the aging effects for the 7-C12-10 and 7-C12-40 polymers. Both terpolymers dissolve completely in deionized water within 24 h. The apparent viscosity of 7-C12-10 decreases only slightly before reaching a constant value within 3 days. A more pronounced aging effect was observed for 7-C12-40. Apparently, the stronger hydrophobic interactions require a longer period of time to reach equilibrium. Furthermore, freshly prepared 7-C12-40 solution exhibits intermolecular associating behavior (Figure 6). In contrast, intramolecular association is observed for the same solution at equilibrium. This indicates that the 7-C12-40 terpolymer undergoes a conformational transition from intermolecular to intramolecular during the aging process. No such conformational transition is observed for 7-C12 terpolymers at lower hydrophobe levels or for all 7-C8 terpolymers.

Effects of Hydrophobic Groups. To assess the effects of hydrophobic monomer content on viscosity behavior in
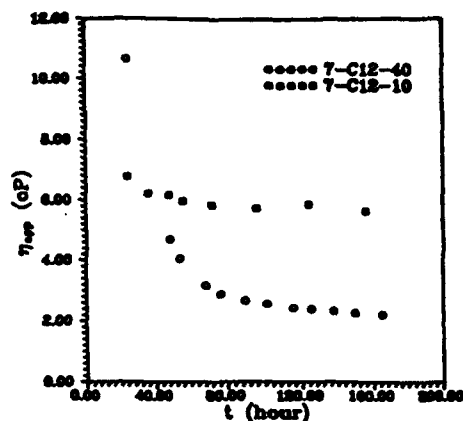

Figure 5. Aging effects of 7-C12-10 and 7-C12-40 terpolymers at 25 °C in deionized water. C_P = 2.0 g/dL. Shear rate: 1.28 s^{-1}.
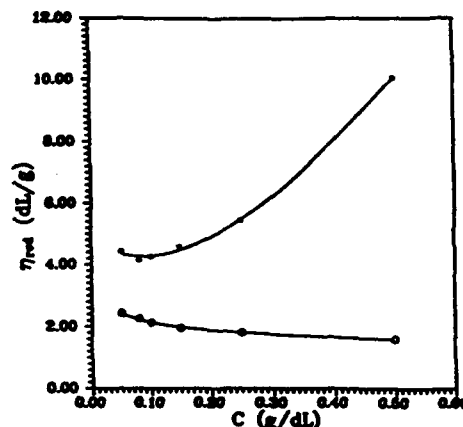

Figure 6. Comparisons of the viscosity behavior of 7-C12-40 terpolymer at 25 °C in deionized water at different times: (●) 1 day after sample preparation; (O) 20 days after sample preparation. Shear rate: 1.28 s^{-1}.
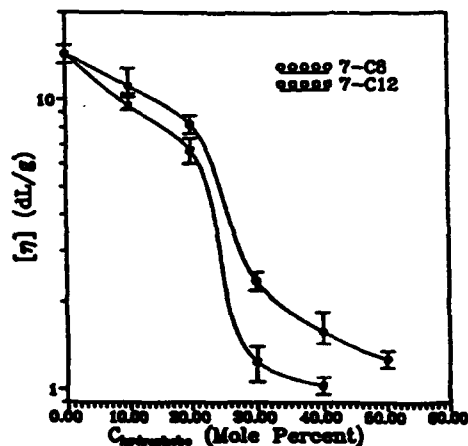

Figure 7. Effects of n-octyl and n-dodecyl group content on the intrinsic viscosity of 7-C8 and 7-C12 terpolymers in deionized water.

deionized water, it was first necessary to determine intrinsic viscosity utilizing the Fuoss relationship.[34] For polymers with largely intramolecular association, the Fuoss plot should yield values which may be used to project molecular weights of single coils. Figure 7 shows the intrinsic viscosities of 7-C8 and 7-C12 terpolymers as a function of composition. The intrinsic viscosities decrease dramatically as the hydrophobe concentration in the terpolymers increases from 0 to 50 mol %. A larger quantity of hydrophobic groups effectively enhances the hydrophobic interactions, resulting in collapse of the polymer coil. A sharp drop in the intrinsic viscosity is
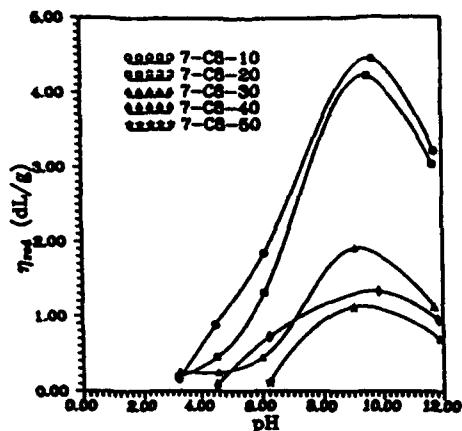
Figure 8. Effect of solution pH on η_{red} for 7–C8 terpolymers at 25 °C. C_P = 0.5 g/dL. Shear rate: 1.28 s⁻¹.

Figure 9. Effect of ionic strength on η_{red} of 7–C8 terpolymers at 25 °C. C = 0.15 g/dL. Shear rate: 1.28 s⁻¹.

Figure 10. Dependence of average lifetime for monomer emission on hydrophobe content.

observed at hydrophobe concentrations between 20 and 30 mol % for both 7–C8 and 7–C12 terpolymers. Similar observations have been reported for other intramolecular associative copolymers[2-5] and have been attributed to a transition from random coil to tighter hypercoil. Dodecyl terpolymers possess a more compact structure than their octyl analogs at constant hydrophobe levels as indicated by the slightly lower intrinsic viscosities of the former (Figure 7).

Effect of pH. The amphiphilic terpolymers contain a large number of carboxyl functional groups as the major hydrophilic component. Variation in pH can impart significant change in solution properties. Figure 8 illustrates the viscosity behavior of the terpolymers containing varying n-octyl concentration at selected pH values. The reduced viscosities of all the polymer solutions initially increase with increasing pH and then decrease. The maximum value of reduced viscosity for all 7–C8 polymers is observed about pH 9.5. Changes in the reduced viscosity are qualitatively similar to those observed for maleic anhydride and alkyl vinyl ether copolymers.[7,9-11] The degree of ionization of the terpolymers increases with increasing pH, disrupting intramolecular associations. The terpolymers reach maximum charge density at the pH at which the highest reduced viscosity is achieved. Further increase in pH increases the concentration of sodium ions in the solution; therefore, the interaction between the charged groups along the polymer backbone is shielded, causing polymer coil collapse.

When the pH of the solution is below the pK_a of the carboxylic acid, most, if not all, of the charges carried by the terpolymers are neutralized. Therefore, there are not enough charged groups on the surface of the polymer coil to prevent macromolecular aggregation and macrophase separation occurs. For example, the terpolymers with 40 and 50 mol % octyl groups precipitate below pH 4 and 5, respectively.

Effect of Electrolyte Addition. The effect of NaCl on the viscosity of the terpolymer solutions was investigated using 7–C8 series (Figure 9). The reduced viscosity decreases for all terpolymers as the NaCl molarity increases due to the shielding of ionic interactions of the carboxylate groups. The terpolymers with high hydrophobe content such as 7–C8–50 precipitate at high salt concentration (~0.5 M).

Effect of Shear Rate. The effect of shear rate on the apparent viscosity of 7–C8–10, 7–C8–30, and 7–C8–50 terpolymers was investigated in pure water at both low and high polymer concentrations. Unlike some of the reported systems which showed shear-thickening behav-
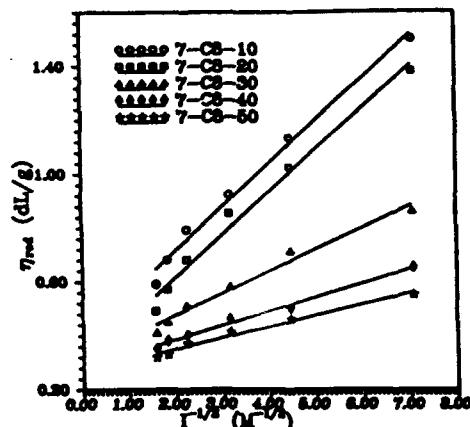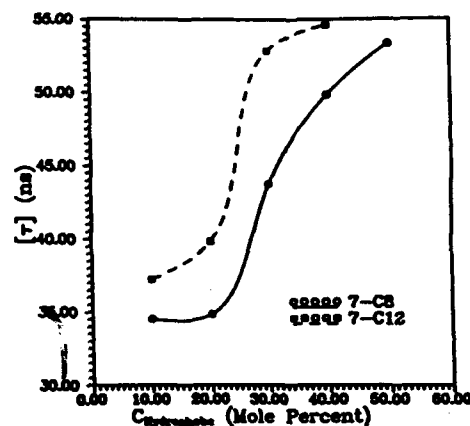
Table 2. Fluorescence Lifetime [τ] (ns) of Monomer Emission Decay for 7–C8 Terpolymers in Aqueous Solutions

pH	7-C8-10	7-C8-20	7-C8-30	7-C8-40	7-C8-50
3	44.3	52.5	57.1	58.8	60.8
5	41.2	44.3	49.1	54.1	58.1
7	34.5	34.8	43.7	49.8	54.3
8	28.9	31.4	40.1	48.5	52.6
10	24.1	28.3	36.6	44.3	51.6

ior,[25] the terpolymers at 0.05 g/dL exhibit Newtonian behavior over the shear rate regime. Similar behavior is observed for the terpolymers at 0.5 g/dL.

Photophysical Studies. Fluorescence measurements were conducted in attempts to evaluate associative properties of the terpolymers in aqueous solutions. Naphthalene lifetimes and excimer to monomer ratios (I_E/I_M) were recorded to monitor the changes of terpolymer conformation.

Effects of Hydrophobic Groups. A significant change in the fluorescence decay of monomer fluorescence (330 nm) is observed upon altering the hydrophobe content (Figure 10). The decay profiles of the terpolymers are complex but can be approximately fit by a sum of two exponentials composed of a longer-lived component and a shorter-lived component. Table 2 summarizes the values of the average lifetime[26] defined by the following equation:

$$[\tau] = \sum a_i \tau_i^2 / \sum a_i \tau_i$$

where a_i is the preexponential factor of the ith component and τ_i is the corresponding lifetime.

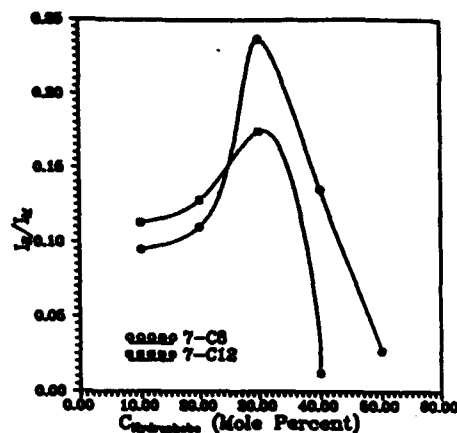Multiexponential fits have been reported for other associative polymers in aqueous media[16,27] and are probably

Figure 11. Dependence of I_E/I_M for both 7-C8 and 7-C12 terpolymers on the hydrophobe content at pH 7.

Figure 12. Dependence of I_E/I_M on solution pH for 7-C8 terpolymers. $C_P = 0.05$ g/dL.

due to labels in different environments contributing to the emission. It is noteworthy in our case that the values of [τ] increase with increasing hydrophobe concentration. The lifetimes of the naphthalene-labeled model compound 4 in solutions of THF, ethanol, methanol, and 50/50 methanol/water are 68.1, 47.2, 30.7, and 21.8 ns, respectively, demonstrating the effect of microenvironmental polarity on the decay of the excited state of the naphthalene label. Therefore, the increase in the average lifetime at higher hydrophobe content is consistent with coil collapse. Also, the 7-C12 series polymers have higher [τ] values than the 7-C8 analogs with the same hydrophobe mole percent. Longer side chains effectively enhance the hydrophobic effect, providing shielding of the naphthyl label from water. These results are similar to those previously reported for hydrophobically modified poly-(allylamine)s.[28]

In addition to changes in the average lifetime, terpolymers also show a dramatic hydrophobe concentration dependence of I_E/I_M. As indicated in Figure 11, I_E/I_M values of the terpolymers increase as the hydrophobe concentration varies from 10 to 30 mol %. Further increases in the hydrophobe concentration result in decreases in I_E/I_M. It is unlikely that excimer formation is due to nearest-neighbor interactions since the number of naphthyl groups are small and they are separated over a large distance along the polymer backbone. The initial increase in I_E/I_M may be attributed to increased compaction of the polymer coil which facilitates the formation of the excimer due to the reduced separation of the chromophore within the hydrophobic microdomain. When the hydrophobe concentration is above 30 mol %, however, the large hydrophobe quantities within the polymer coil separate the naphthyl labels. Furthermore, the highly compact hydrophobic microdomains limit the mobility of the chromophores, preventing orientation in a manner favorable for excimer formation. The latter effect has been observed previously by our group[16] and elsewhere[29] for naphthalene-labeled methacrylic acid copolymers. The negligible formation of excimer in 7-C8-50 and 7-C12-40, which have high hydrophobe concentrations, lends credence to these arguments.

Effect of pH. The effect of solution pH on the conformation of the terpolymers was first examined by the lifetime measurement at 330 nm of the monomer decay of 7-C8 systems. Decrease in [τ] indicates the expansion of the hydrophobic domains with increasing pH (Table 2). The highly hydrophobic terpolymers such as 7-C8-40 and 7-C8-50 remain in the rather compact conformations at high pH as evidenced by the large [τ] values.
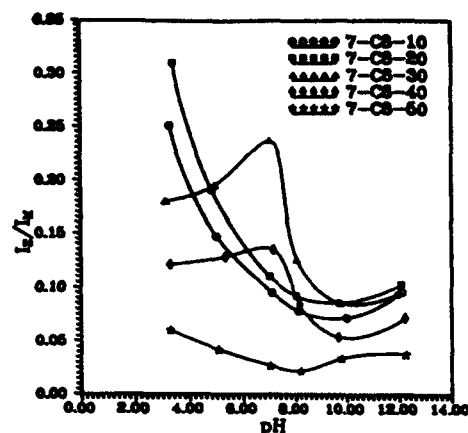
Figure 12 depicts the pH dependence of I_E/I_M for 7-C8 polymers in the aqueous solutions. For the 7-C8-10 and 7-C8-20 polymers, I_E/I_M decreases in the pH range 3-10 and then increases slightly at higher pH. This is consistent with the rheological response of the terpolymers. As the pH increases, the polymer coil expands and the naphthyl groups are separated, leading to decreased I_E/I_M. The lowest I_E/I_M occurs at the pH where maximum expansion of the polymer coil occurs. A slight rise in I_E/I_M at higher pH is attributed to an increase in ionic strength. 7-C8-20 exhibits a higher I_E/I_M value at a given pH than does 7-C8-10 due to a more compact structure of the former.

The 7-C8-30 polymer exhibits a different dependence of I_E/I_M on the pH. The curve first rises and then drops rapidly upon increasing the pH. The initial increase in the values of I_E/I_M may be explained by increased mobility of the naphthyl groups in the hydrophobic microdomains upon coil expansion. At pH 3, the terpolymer adopts a highly collapsed conformation, indicated by the very low reduced viscosity. The mobility of the chromophore is largely restricted. The probability of two chromophores approaching each other in a coplanar or sandwich manner, a necessity for the excimer formation, is small. As the pH is increased, chain expansion decreases polymer coil density. The relatively large separation of the naphthyl moieties from the polymer backbone allows the chromophores to maintain proximity to one another upon moderate expansion; this, coupled with increased mobility of the chromophores, leads to increased I_E/I_M. Further increase in the coil size at higher pH, however, results in long-range separation of the chromophores and I_E/I_M decreases rapidly. This behavior is significantly different from that observed previously by our group[16] for naphthalene-labeled poly(methacrylic acid). In that case, I_E/I_M increased rapidly at a pH of 7.5 but exhibited no decrease thereafter. Relatively large quantities of naphthyl labels and long spacers in that copolymer were reported to allow association even in a highly expanded coil. For the 7-C8-30 terpolymer, however, each naphthyl group is surrounded by comparatively large neighboring octyl groups upon coil expansion,[6,7] resulting in a decrease in I_E/I_M. Interestingly, the I_E/I_M maximum is observed at lower pH than the reduced viscosity maximum (Figure 8), indicating that significant chromophore separation occurs before the polymer chain reaches its maximum expansion.

The terpolymer 7-C8-40 also shows an increase in I_E/I_M, but to a lesser extent. This can be attributed to the increased difficulty of two chromophores approaching each other in a large hydrophobe concentration within the

Table 3. Fluorescence Lifetime [τ] (ns) of Monomer Emission Decay for 7-C8 Terpolymers in NaCl Aqueous Solutions

C_{NaCl} (M)	7-C8-10	7-C8-20	7-C8-30	7-C8-40	7-C8-50
0.1	49.3	54.5	60.1	65.3	68.6
0.2	55.2	58.7	64.5	67.6	69.5
0.3	58.9	62.4	67.5	69.9	72.4
0.4	61.2	63.5	69.8	71.3	74.1

polymer coil. This is consistent with the observation that the 7–C8–40 polymer always exhibits a lower I_E/I_M value than 7–C8–30 at a constant pH.

Values of I_E/I_M for 7–C8–50 change little with pH. In this case, very high hydrophobe concentration within the polymer coil results in almost complete diminution of the naphthyl group interactions. Each naphthyl label is surrounded by a large number of octyl groups even in the expanded coil. Therefore, low I_E/I_M values are observed throughout the pH range.

Effect of Electrolyte Addition. Table 3 lists the average lifetimes of the monomer emission for 7–C8 polymers at different NaCl concentrations. Increased average lifetime is consistent with the compaction of the polymer coil with increasing salt concentration. The terpolymers with higher hydrophobe content are less sensitive to the ionic strength as indicated by the reduced curvature of the data. The reduced sensitivity may be explained by the already very compact conformation adopted by the terpolymers in the salt-free solution as well as the decreased quantity of charged groups along the terpolymer chain.

Conclusions

A series of naphthalene-labeled poly(maleic anhydride-alt-ethyl vinyl ether) copolymers containing pendent octyl and dodecyl groups have been synthesized. The fluorescence and viscosity behavior of the terpolymers have been studied as a function of the length and concentration of the hydrophobic groups, electrolyte concentration, and the solution pH. In all cases investigated, the terpolymers exhibit a tendency toward intramolecular association in dilute and semidilute aqueous solutions at specifically varied conditions of pH and ionic strength. The terpolymers with longer alkyl groups and/or higher alkyl group content tend to be more compact and exhibit lower viscosity in deionized water. Addition of either acid or base results in decreased solution viscosity. Addition of NaCl lowers significantly the viscosity of the polymer solutions, indicating a more collapsed conformation at higher ionic strength. Photophysical data parallel the rheological responses. I_E/I_M values of naphthyl moieties from steady-state fluorescence increase initially, reach a maximum, and then decrease upon increasing hydrophobe concentration from 10 to 50 mol %. Extended lifetime of the monomer fluorescence decay with increasing hydrophobe level indicates increased compaction of the polymer coil. As the pH values change from 3 to 10, significant decreases in the I_E/I_M values are observed for the ter-

polymers with low hydrophobe content due to coil expansion. I_E/I_M values of the terpolymers with moderate hydrophobe content rise initially and then decrease continuously over the same pH range. Systems with high hydrophobic content show little dependence on pH. These results are explained by considering the relative concentration of the chromophore in the hydrophobic microdomains as well as the change in the mobility of the naphthyl moieties with the compactness of the polymer coils. The lifetime of the monomer emission decreases with increasing pH, indicating a more hydrated environment experienced by the probe within the polymer coil.

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Water-Soluble Polymers. 60. Synthesis and Solution Behavior of Terpolymers of Acrylic Acid, Acrylamide, and the Zwitterionic Monomer 3-[(2-Acrylamido-2-methylpropyl)dimethylammonio]-1-propanesulfonate

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ABSTRACT: Terpolymers of acrylic acid (AA), acrylamide (AM), and the zwitterionic monomer 3-[(2-acrylamido-2-methylpropyl)dimethylammonio]-1-propanesulfonate (AMPDAPS) have been prepared by the free-radical polymerization in a 0.5 M NaCl aqueous solution using potassium persulfate as the initiator. The feed ratio of AMPDAPS:AA:AM was varied from 5:5:90 to 40:20:20 mol %, with the total monomer concentration held constant at 0.45 M. Terpolymer compositions were obtained by ^{13}C NMR. Low-angle laser light scattering provided molecular weights and second virial coefficients which varied from (3.0 to 7.9) $\times 10^6$ and (2.23 to 2.95) $\times 10^{-4}$ mL mol g^{-2}, respectively. The solubilities of the resulting terpolymers are dependent on pH as well as the amount of AMPDAPS and AA present in the feed. At pH 4 and for higher incorporation of AA and AMPDAPS in the feed (>25 mol %), the terpolymers are insoluble in deionized water and 0.25 M NaCl. At pH 8, all terpolymers are soluble in deionized water and salt solutions. The dilute and semidilute solution behavior of the terpolymers were studied as a function of composition and added electrolytes. Polyelectrolyte behavior was observed for all terpolymers at pH 8 as evidenced by a viscosity decrease in the presence of added electrolytes. The terpolymers exhibit higher viscosities in the presence of NaSCN versus NaCl. Comparison of the solution behavior of the terpolymers to copolymers of AM and AA as well as copolymers of AMPDAPS and AM has been made.

Introduction

The synthesis of electrolyte-tolerant water-soluble polymers that contain ionic pendent groups has been the subject of study in our laboratories for the past several years.[1-8] Polyelectrolytes display high viscosities in deionized water at low concentrations and thus are commercially used as viscosifying agents in numerous applications. However, in the presence of added electrolytes, charge–charge repulsions are shielded, resulting in a considerable decrease in viscosity and, in some cases, phase separation. In striving to synthesize polymers that show tolerance in the presence of added electrolytes, we have recently focused our attention on polyampholytes, polymers which possess both cationic and anionic pendent groups. Polyampholytes have shown an enhancement in viscosity in the presence of added electrolytes as intramolecular Coulombic attractions are shielded.[9-21] In our laboratories, high charge density copolymers of cationic and anionic monomers have been synthesized. The rheological behavior of these polymers is dictated by the charge ratio of the cationic and anionic groups. If there is a charge imbalance, the polymers behave as polyelectrolytes and display a decrease in viscosity in the presence of added electrolytes. However, when there are equimolar anionic and cationic monomers, polyampholytic behavior is observed and viscosity increases as the ionic strength of the medium is increased. Low charge density polyampholytes have also been synthesized in which a neutral monomer such as acrylamide has been terpolymerized with cationic and anionic monomers. These polymers intermolecularly associate in deionized water and can form gels that may be used as "superabsorbers".[17]

Polyampholytes have also been synthesized from zwitterionic monomers in which the cationic charge is provided by a quaternary ammonium functionality and the anionic charge is provided by a carboxylate or sulfonate group. These monomers are unique in that they exhibit a net charge of zero at appropriate pH. A number of investigations have focused on the unusual solution behavior of zwitterionic polyampholytes.[22-34]

The goals of this research are to synthesize polymers that will display either polyampholytic or polyelectrolytic behavior depending on the pH of the environment. Special attention has been focused on polymers that contain the carboxylic acid group which may be neutralized to the carboxylate group by adjusting the pH of the medium. Thus, the synthesis and solution behavior of terpolymers of acrylic acid, acrylamide, and a sulfobetaine monomer will be discussed.

Experimental Section

Materials. Acrylamide (AM) from Aldrich was recrystallized twice from acetone and vacuum-dried at room temperature. Acrylic acid (AA) from Aldrich was distilled prior to use. 3-[(2-Acrylamido-2-methylpropyl)dimethylammonio]-1-propanesulfonate (AMPDAPS) was synthesized by the ring-opening reaction of 1,3-cyclopropanesultone with (2-acrylamido-2-methylpropyl)dimethylamine as previously reported.[19] Potassium persulfate from J. T. Baker was recrystallized twice from deionized water. All other materials were used as received.

Synthesis of Terpolymers of 3-[(2-Acrylamido-2-methylpropyl)dimethylammonio]-1-propanesulfonate, Acrylamide, and Acrylic Acid. The terpolymers of AMPDAPS, AM, and AA (the AADAPS series) were synthesized by free-radical polymerization in a 0.5 M NaCl aqueous solution under nitrogen at 30 °C using 0.1 mol % potassium persulfate as the initiator at a pH of 8. The use of 0.5 M NaCl as a reaction medium was to keep consistent with methods employed in ref 19. The feed ratio of AMPDAPS:AA:AM was varied from 5:5:90 to 40:40:20 mol %, with the total monomer concentration held constant at 0.45 M.

In a typical synthesis, specified quantities of each monomer were dissolved in small volumes of a 0.5 M NaCl solution. The separate solutions were then combined and diluted to a 0.45 M monomer concentration with a 0.5 M NaCl solution. One

equivalent of sodium hydroxide per equivalent of acrylic acid was then added and the pH adjusted to 8. The reaction mixture was sparged with nitrogen and then initiated with 0.1 mol % potassium persulfate. The reaction was usually terminated at <55% conversion due to the high viscosity of the reaction medium and as a precaution against copolymer drift. The polymers were precipitated in acetone, redissolved in deionized water, and then dialyzed using Spectra/Por 4 dialysis bags with molecular weight cutoffs of 12 000–14 000. Half of the samples were dialyzed against deionized water adjusted to pH 4, while the other half were dialyzed against deionized water adjusted to pH 8. Polymers containing 25 mol % or more of AMPDAPS and AA in the feed (AADAPS-25 and AADAPS-40) precipitated during dialysis against deionized water adjusted to pH 4. After dialyzing for 2 weeks, the polymers were isolated by lyophilization. Conversions were determined gravimetrically. IR: AADAPS-40 copolymer isolated at pH 4 (KBr pellet): 3600–2600 (b d, $O–H$), 3260 (s, $N–H$), 3050, 2980 (m, $C–H$), 1700, 1655, 1637 $C=O$), 1560 (m, $N–H$), 1460 (m, $C–O–H$), 1208 (s, $C–O$), 1189, 1042 cm^{-1} (s, $S–O$).

Synthesis of Copolymers of 3-[(2-Acrylamido-2-methylpropyl)dimethylammonio]-1-propanesulfonate with Acrylamide and Acrylic Acid with Acrylamide. The polymerization procedures for copolymers of AMPDAPS with AM have been previously reported by Salazar and McCormick.[19] Copolymers of AM and AA were prepared using similar techniques for the terpolymer synthesis.

Copolymer Characterization. ^{13}C NMR spectra of the polymers were obtained at 50.3 MHz on a Bruker AC 200 spectrometer using 10–15 wt % aqueous (D_2O) polymer solutions with DSS as a reference. A recycle delay of 6 s, 90° pulse length, and gated decoupling to remove all NOE were used for quantitative spectral analysis. FTIR spectra were obtained using a Mattson Galaxy 2020 series spectrometer. Molecular weight studies were performed on a Chromatix KMX-6 low-angle laser light scattering instrument. Refractive index increments were obtained using a Chromatix KMX-16 laser differential refractometer. For quasielastic light scattering a Langley–Ford Model LF1-64 channel digital correlator was used in conjunction with the KMX-6. All measurements were conducted at 25 °C in 1 M NaCl at a pH of 8.

Viscosity Measurements. Stock solutions of sodium chloride were prepared by dissolving the appropriate amount of salt in deionized water adjusted to either pH 4 or pH 8 in volumetric flasks. Polymer stock solutions were made by dissolving amounts of polymer in the salt solutions. The solutions were then diluted to appropriate concentrations and allowed to age for 7–10 days before being analyzed with a Contraves LS-30 rheometer. Intrinsic viscosities were evaluated using the Huggins equation.

Results and Discussion

Compositional Analysis. The terpolymers of AMPDAPS, AM, and AA (the AADAPS series) were synthesized by varying the feed ratios of AMPDAPS:AA:AM from 5:5:90 to 40:40:20 mol %. Copolymers of AM and AA (the AAAM series) were synthesized by varying the feed ratio of AA:AM from 10:90 to 25:75 mol %. Copolymers of AMPDAPS and AM (the DAPSAM series) have been synthesized previously.[19] All polymers reported in this paper are shown in Chart 1. The polymer compositions were determined by integration of the carbonyl resonances and agree favorably with past results. A typical NMR spectrum is shown in Figure 1. AMPDAPS and AM incorporation in both the AADAPS and DAPSAM series approximates the feed composition, indicating little preference for addition of either monomer. AA incorporation in both the AADAPS and AAAM series was found to be approximately half the feed composition, denoting a partiality toward the addition of the acrylamido-type monomers. The reaction parameters and the resulting copolymer compositions are given in Table 1.

Low-Angle Laser Light Scattering. Table 2 shows the weight-average molecular weights determined by low-angle laser light scattering at 25 °C in 1 M NaCl. The
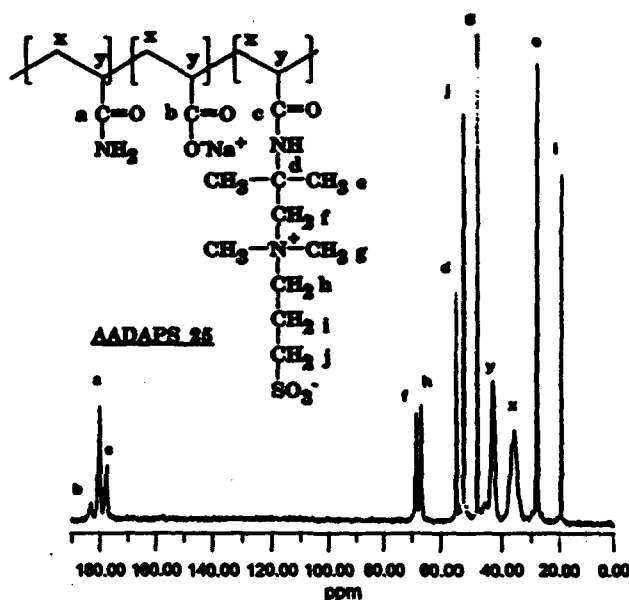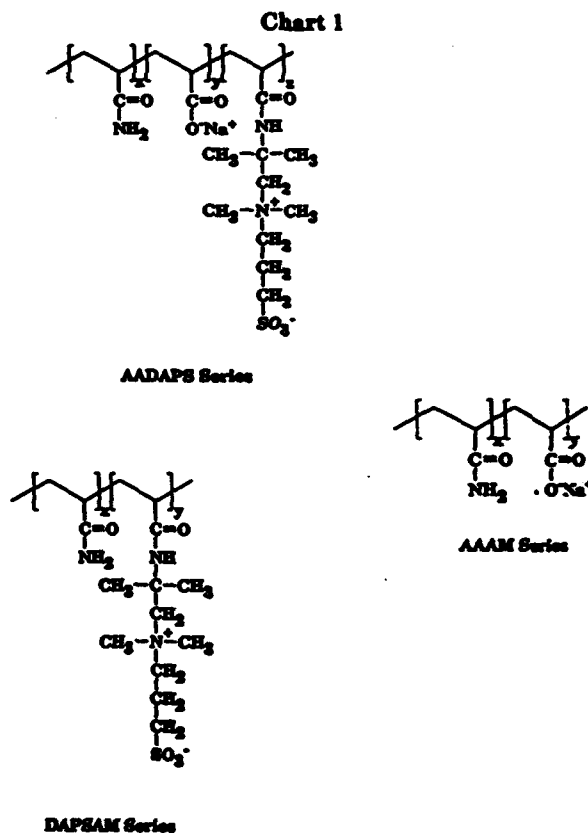

Figure 1. ^{13}C NMR of AADAPS-25.

Chart 1

molecular weight for the AADAPS series varies from (3.0 to 7.9) × 10^6. The second virial coefficients (A_2) for the AADAPS series have values between those of the DAPSAM series and the AAAM series. This is expected due to the presence of the strongly hydrated carboxylate functionalities in the AADAPS terpolymers.

Quasi-elastic light scattering was used to obtain the mean diffusion coefficients (D_0) and diameters (d_0) shown in Table 2. As molecular weight and degree of polymerization increase, D_0 decreases and d_0 increases on average although solvent quality (A_2 values) as well as steric factors must also be considered. AADAPS-25 and DAPSAM-25, both having similar molecular weights, demonstrate enhanced solvation of AADAPS-25 due to the presence of carboxylate functionalities, resulting in a larger hydrodynamic volume.

Table 1. Reaction Parameters for AADAPS, AAAM, and DAPSAM Polymers

Reaction Parameters for AADAPS Terpolymers

sample	feed ratio (mol %) (X/Y/Z)	reaction time (h)	conv (%)	found[a] (mol %) AMPDAPS	AA	AM
AADAPS-5	90.0/5.0/5.0	4.0	53.0	5.0	3.2	91.8
AADAPS-10	80.0/10.0/10.0	4.0	48.0	10.2	4.6	85.2
AADAPS-25	50.0/25.0/25.0	4.0	46.0	31.1	13.3	55.6
AADAPS-40	20.0/40.0/40/0	5.5	50.0	50.4	25.4	24.2

Reaction Parameters for AAAM and DAPSAM Copolymers

sample	feed ratio (mol %) (X/Y)	reaction time (h)	conv (%)	found[a] (mol %) AMPDAPS	AA	AM
AAAM-10	90.0/10.0	4.0	65.0		5.8	94.2
AAAM-25	75.0/25.0	6.0	38.0		12.2	87.8
DAPSAM-10[b]	90.0/10.0	4.5	22.1	9.6		90.4
DAPSAM-25[b]	75.0/25.0	23.0	44.9	27.7		72.3

[a] Determined from ^{13}C NMR. [b] Reference 19.

Table 2. Classical and Quasi-Elastic Light Scattering Data for AADAPS, AAAM, and DAPSAM Polymers

sample no.	dn/dc	M_w ($\times 10^{-6}$)	A_2 ($\times 10^4$ mL mol g^{-2})	$D_0 \times 10^8$ (cm^2/s)	d_0 (Å)	DP $\times$ 10^{-4}
AADAPS-5	0.186	3.0	2.77	4.07	1150	3.61
AADAPS-10	0.175	5.4	2.95	4.10	1150	5.69
AADAPS-25	0.193	7.9	2.23	3.91	1370	5.55
AADAPS-40	0.181	4.7	2.23	4.00	1180	2.50
AAAM-10	0.172	4.0	5.76	4.09	1180	5.49
AAAM-25	0.189	1.9	9.49	4.12	1060	2.53
DAPSAM10	0.138	7.00	1.49	4.01	1380	7.50
DAPSAM25	0.139	8.20	1.33	3.97	1180	6.35

Dilute Solution Behavior. Effects of Copolymer Composition and pH. To study the effects of pH on the terpolymers, 0.25-g/dL stock solutions were prepared from the terpolymer samples dialyzed at pH 4 and pH 8. Of the samples dialyzed against pH 4, only two of the samples were soluble in deionized water: AADAPS-5 and AADAPS-10. AADAPS-25 was insoluble in deionized water and 0.25 M NaCl; however, the copolymer was soluble in 1 M urea. AADAPS-40 was insoluble in all of the above solvents.

The solubility behavior is a culmination of two effects: (1) charge–charge interaction between the AMPDAPS mer units and (2) hydrogen bonding between the amide and carboxylic acid units. The insolubility of polyampholytes in deionized water has been observed numerous times and is clearly due to strong Coulombic attractions. In our group, copolymers of AM and AMPDAPS have been studied.[19] Those copolymers containing 60 mol % or more of AMPDAPS were insoluble in water and required the addition of a critical concentration of NaCl before solubility could be achieved. In the case of AADAPS terpolymers, the solubility is greatly reduced by substituting acrylamide units with acrylic acid units during synthesis.

The behavior of acrylamide/acrylic acid copolymers has been studied by numerous groups.[36-41] Precipitation has been observed for polymers containing more than 30 mol % acrylic acid in 0.05 M HCl due to strong hydrogen bonding between the acid and amide groups.[36] Similar behavior has been observed in AADAPS terpolymers and is further supported by the solubilization of AADAPS-25 which requires 1 M urea. The presence of urea may disrupt hydrogen bonding between the carboxylic acid and amide groups and allows solubilization of the terpolymer.

Although polymer samples AADAPS-5 and AADAPS-10 were soluble in deionized water as well as salt solutions, viscosity data yielded little information due to the
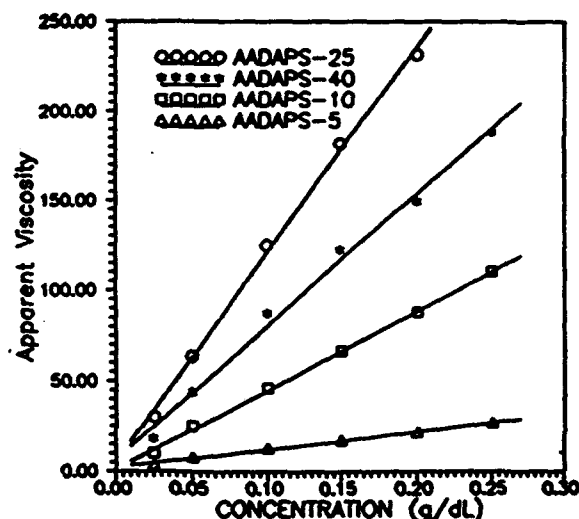

Figure 2. Apparent viscosities of AADAPS terpolymers in deionizing water. (Determined at 25 °C at a shear rate of 5.96 s^{-1} and pH 8.)

extremely low values that were obtained (η_{red} < 0.5). Extremely compact conformations are indicated in both deionized water and 0.514 M NaCl. As for the samples dialyzed against pH 8, all were soluble in both deionized water and salt solutions.

Effects of Added Electrolytes on the AADAPS Series. The effects of added salts on the viscosities of the AADAPS terpolymers were observed at a shear rate of 5.96 s^{-1}. The apparent viscosities of the AADAPS terpolymers in deionized water at pH 8 are shown in Figure 2. As expected, the terpolymers display high viscosities due to a net negative charge on the chain which causes the polymer to behave as a polyelectrolyte. Viscosity tends to increase with increasing sodium acrylate mer units except for the discrepancy between AADAPS-40 and AADAPS-25, perhaps due to molecular weight differences or increased rigidity from cyclic interactions between amide and carboxylate groups. Our group and others have found that the highest intrinsic viscosities for copolymers of acrylamide and sodium acrylate occur when there is roughly an equal ratio of acrylamide to sodium acrylate units due to the cyclization mechanism noted above.[35,36] This nearest-neighbor effect may be broken up by the AMPDAPS mer units in AADAPS-40 which contains 50 mol % AMPDAPS.

The AADAPS terpolymers act as anionic polyelectrolytes with a reduction in the intrinsic viscosity as a function
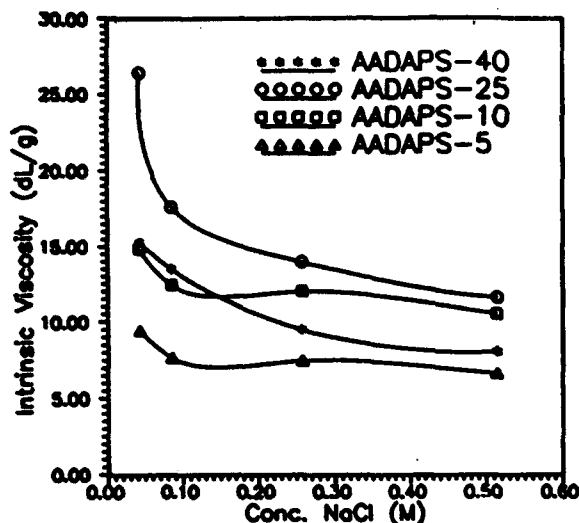
Figure 3. Dependence of intrinsic viscosity of AADAPS terpolymers as a function of NaCl concentration. (Determined at 25 °C at a shear rate of 5.96 s⁻¹ and pH 8.)
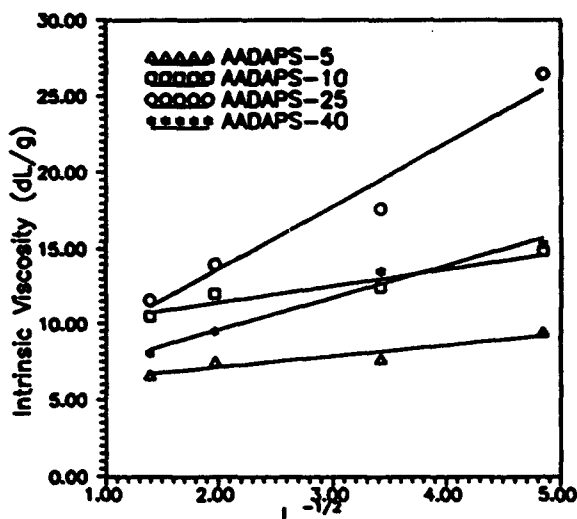

Figure 5. Reduced viscosity of AADAPS terpolymers versus NaCl concentration with a polymer concentration of 0.25 g/dL. (Determined at 25 °C at a shear rate of 5.96 s⁻¹ and pH 8.)

Figure 4. Intrinsic viscosities of AADAPS terpolymers as a function of the inverse square root of ionic strength. (Determined at 25 °C at a shear rate of 5.96 s⁻¹ and pH 8.)

Figure 6. Reduced viscosity as a function of increasing ionic strength of various salts with a polymer concentration of 0.25 g/dL. (Determined at 25 °C at a shear rate of 5.96 s⁻¹ and pH 8.)

of increasing ionic strength as shown in Figure 3. As the concentration of NaCl increases, the anionic repulsions are shielded and the hydrodynamic volume decreases as the polymer chain relaxes into a more compact configuration. AADAPS-40 displays a lower intrinsic viscosity than AADAPS-10 at higher ionic strengths, probably indicative of a lower molecular weight. Polyelectrolyte behavior was further illustrated by plotting intrinsic viscosity as a function of the reciprocal square-root of the ionic strength (Figure 4). All samples display a linear dependence which is typical of polyelectrolytes. AADAPS-25 exhibits the highest slope and is, therefore, the most sensitive to changes in the ionic strength of the solution.

Figure 5 shows the reduced viscosity versus NaCl concentration at a polymer concentration of 0.25 g/dL. Once again typical polyelectrolyte behavior is observed even up to 3 M NaCl. Polyampholytes normally display an enhancement in viscosity as the ionic strength of the medium increases due to the shielding of intramolecular Coulombic attractions. As the ionic strength of the medium is increased, the electrostatic energy term plays a smaller and smaller role in the conformational stability of the chain while the rotational and hydrophobic terms as well as polymer solvation become the dominant factors in dictating the conformation of the polymer chain. The
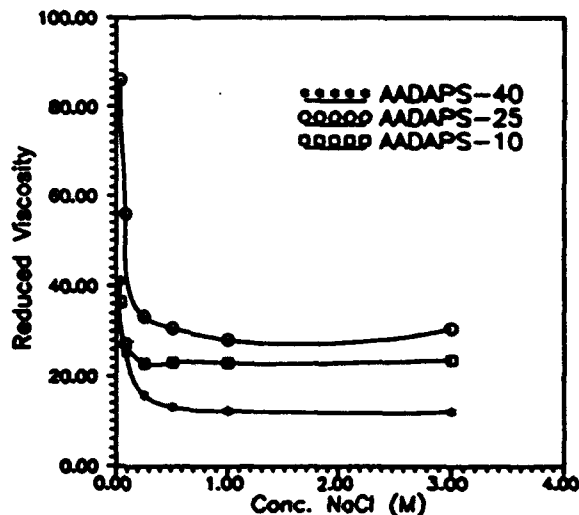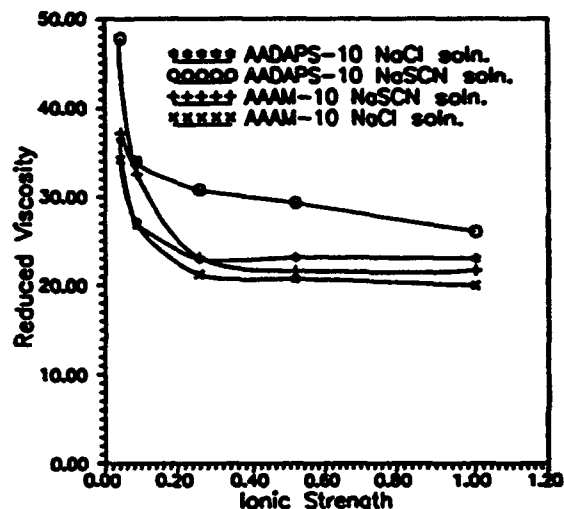
AADAPS terpolymers show no polyampholytic behavior at higher ionic strengths, and the hydrodynamic volumes of the polymers appear to remain fairly constant between 0.5 to 3 M NaCl.

The reduced viscosities were also examined as a function of the structure of the anion in the added electrolytes. Figures 6 and 7 display the differences in the reduced viscosity in NaCl and NaSCN solutions for AADAPS-10, AAAM-10, and AADAPS-25, AAAM-25. In agreement with the Hoffmeister series predictions, all polymers studied show an enhancement of reduced viscosity in NaSCN solutions compared to NaCl solutions; however, the effect is much less dramatic for AAAM copolymers. As noted by others[31-33] and in accord with the "hard-soft" acid-base theory, the SCN⁻ ion is a "softer" ion than the Cl⁻ ion and is thus able to bind tighter to the "soft" ammonium group. Salamone et al.[31] have proposed that tighter binding between the small anion and the ammonium group in sulfobetaine polymers reduces the draining ability of individual chains and thus an increase in viscosity is observed. It is further stated that, as the concentration of salt is increased, the viscosity rises rapidly at first due to site binding and then increases slowly as atmospheric binding becomes the only mode available for
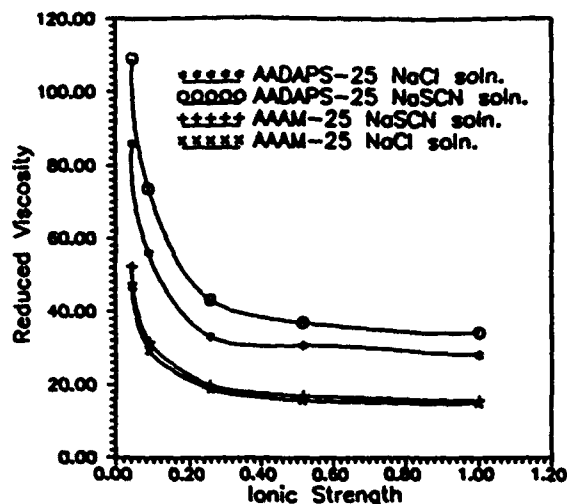
Figure 7. Reduced viscosity as a function of increasing ionic strength of various salts with a polymer concentration of 0.25 g/dL. (Determined at 25 °C at a shear rate of 5.96 s⁻¹ and pH 8.)

Figure 8. Dependence of intrinsic viscosity as a function of NaCl concentration. (Determined at 25 °C at a shear rate of 5.96 s⁻¹ and pH 8.)

Figure 9. Dependence of intrinsic viscosity as a function of NaCl concentration. (Determined at 25 °C at a shear rate of 5.96 s⁻¹ and pH 8.)

ionic interaction. Liaw et al.[33] suggested that, if the small anion binds tighter to the ammonium group than the small cation does to the sulfonate group in sulfobetaine polymers, a partial negative charge may develop along the polymer chain and thus lead to an increase in viscosity.

Our systems show that, at the lower ionic strengths, the differences in reduced viscosity between the NaSCN and NaCl solution are quite large. However, at higher salt concentrations the differences begin to narrow. This behavior is in agreement with the propositions put forth by Salamone and Liaw.[31,33] At lower concentrations of salt, the SCN⁻ anion binds tighter to the ammonium group than does the Cl⁻ anion, leading to an enhancement of the reduced viscosity. This "tighter" binding not only reduces the draining ability of the polymer chain but leads to an amplification of the net negative charge that is already present along the polymer chain due to the sodium acrylate mer units. At higher concentrations of salt, electrostatic repulsive forces are greatly diminished and the differences in viscosity between the NaCl and the NaSCN solutions are probably due to draining differences of the polymer in the different solutions as well as the enhanced "salting-in" of the polymer chain by NaSCN.

Comparison of the Effect of Added Electrolytes on the AADAPS, DAPSAM, and AAAM Series. Figures 8 and 9 examine the effect of increasing NaCl concentration on the intrinsic viscosities of all the polymer series. In Figure 8, a structural comparison between AADAPS-10 and AAAM-10 may tentatively be drawn since both have similar molecular weights and degrees of polymerization. It can be seen that, at low ionic strength, the pure polyelectrolyte has a higher intrinsic viscosity due to Donnan effects that have not been overcome. AADAPS-10 has a lower intrinsic viscosity because of Columbic attractions between the cationic and anionic groups present along the polymer chain. When the ionic strength increases, the Columbic attractions in AADAPS-10 are shielded sufficiently enough for the copolymer to adopt a more random configuration; however, there is still a decrease in the intrinsic viscosity due to the charge imbalance of the polymer. At higher ionic strengths there is a crossover of intrinsic viscosity values of the AADAPS-10 and AAAM-10 polymers, with AADAPS-10 maintaining a higher intrinsic viscosity. This behavior is probably a result of the inherent bulkiness of the AMDPAPS mer unit restricting the rotational freedom of the polymer chain
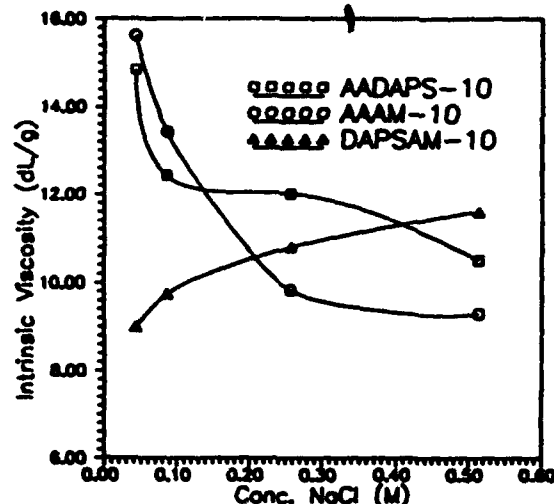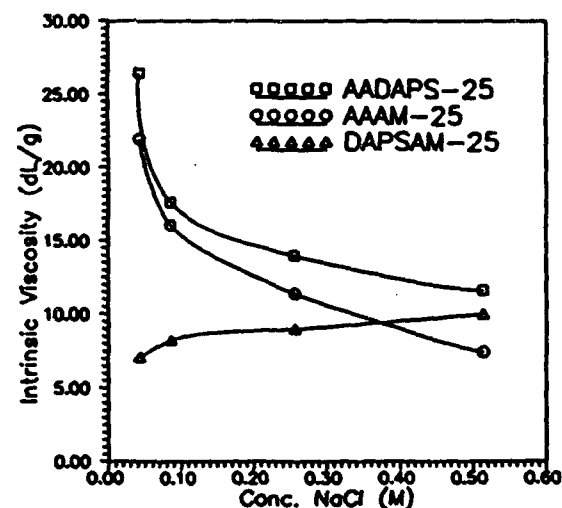
rather than a solvation effect. DAPSAM-10 displays typical polyampholyte behavior, with the intrinsic viscosity increasing in the presence of added salt. DAPSAM-10 has the highest intrinsic viscosity in 0.5 M NaCl, likely due to the higher molecular weight compared to the other polymers. In Figure 9, a structural comparison between AADAPS-25 and DAPSAM-25 may tentatively be drawn since molecular weights and degrees of polymerization are similar. At low ionic strengths, AADAPS-25 has a higher intrinsic viscosity due to the charge imbalance of the polymer chain. At higher ionic strengths, AADAPS-25 still maintains a higher intrinsic viscosity than DAPSAM-25 due to the presence of the carboxylate functionalities which enhance the solvation of the polymer chain. This is in accord with the A_2 values discussed earlier.

Effects of Shear Rate. The effects of shear rate on the apparent viscosity of AADAPS-10 and AADAPS-25 were investigated in deionized water at both high and low polymer concentrations. The shear rates ranged from 0.94–127.47 s⁻¹. In Figure 10, the behavior of the terpolymers is shown at two different concentrations. Both terpolymers display pseudoplastic behavior as evidenced by the reduction in the apparent viscosity as shear rate increase. In 0.514 M NaCl, the terpolymers no longer
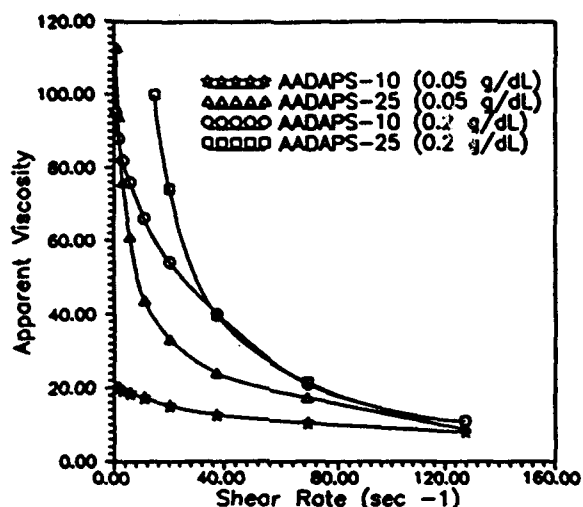
Figure 10. Effect of shear rate on the apparent viscosity of AADAPS-10 and AADAPS-25 in deionized water and different concentrations. (Determined at 25 °C and pH 8.)

display pseudoplastic behavior and viscosity is fairly constant over the range of shear rates studied.

Conclusions

Terpolymers of AA, AM, and AMPDAPS have been synthesized by free-radical polymerization in 0.5 M NaCl. In deionized water at pH 4, the solubility of the polymers is greatly reduced due to ionic interactions as well as hydrogen-bonding interactions. At pH 8, the polymers behave as typical polyelectrolytes displaying high apparent viscosities. As the ionic strength of the medium is increased, the intrinsic viscosity decreases due to shielding effects between like charges. The reduced viscosities are higher in NaSCN solutions compared to the NaCl solutions due to the higher binding ability between the SCN⁻ anion and the ammonium group on the AMPDAPS mer unit. AADAPS-10 displays a higher intrinsic viscosity than AAAM-10 in 0.5 M NaCl probably due to steric factors. AADAPS-25 displays a higher intrinsic viscosity due to solvation effects.

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Design and Synthesis of *Denovo* Hydrophobically Associating Polypeptides

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Introduction

The use of recombinant DNA techniques to produce polymeric materials with a preordained microstructure has met with an encouraging degree of success.[1-3] Likewise, much success in the *denovo* design of a-helical polypeptides[4-5] has provided the impetus for our current research. The goal of this research is to utilize these techniques to produce polymeric materials that form environmentally sensitive reversible hydrophobic interactions. A group of naturally occurring proteins known as exchangeable apolipoproteins, which possess many of these properties, affords us with a well documented model system upon which our current polypeptide sequence is based.

Model Systems

The exchangeable apolipoproteins, among many things, assist in the solubilization of hydrophobic lipid particles in the aqueous environment of an organisms blood, hemolymph, etc. These proteins commonly possess amphipathic a-helices, consisting of an a-helix with opposing polar and non-polar faces aligned along the long axis of the helix.[6] Many of these proteins possess multiple 11 amino acid tandem repeats which demonstrate the amino acid

periodicity associated with amphipathic alpha-helices. The interaction of the helical portions of these proteins is also aided by the distribution of charged amino acids along the polar helix face. The negatively charged amino acids are found to lie approximately in the middle of the polar face, while the positively charged amino acids are positioned towards the outer portion of the polar face. This arrangement maximizes favorable electrostatic interactions with the phospholipids of the lipid particle. In several of these proteins regions that behave as "hinges" have been postulated.[7-8.] It is believed that the function of these regions is to facilitate a rearrangement of the apolipoprotein secondary structure elements in order to regulate the composition of lipoprotein particles.

Polypeptide Design

The polypeptide, designated DN3, consists of the following amino acid sequence:

(DDDPGDLEELLNKLNELLKELNELLKKG G)-

This polypeptide incorporates several specific design considerations in order to maximize helical stability under a variety of conditions. The amino acids utilized for the helical portion of the sequence reflects the occurance of particular residues in naturally occurring a-helices[9] The placement of leucine residues at positions a,d and e of a heptad repeat has been shown to give rise to a "knob and hole" type of interaction between helices. This placement of leucines also produces a distinctly hydrophobic face along the longitudinal axis of the helix. The placement of distinctly acidic and basic residues at the amino and carboxyl termini respectively, has been shown to stabilize the formation of a-helices via favorable interaction with the helical dipole moment. The placement of charged residues in an i,i+4 spacing has also been shown to provide

additional stabilization to the helix via the formation of salt bridges. Glycine and proline residues are utilized to terminate and initiate, respectively, a-helix formation. Asparagine residues have also been used with in the helix so that at a later time the influence of increase negative charge, via deamidation reactions, may be assessed on the polypeptides physical properties. The sequence consisting of -(GGDDDPG--)- is designed to act as an ionizable "hinge" region. It is our intent to use this "hinge" sequence as the starting point from which structure-property relationships may be established concerning the ionization state of this sequence and the over all arrangement of the helical portions of this polypeptide.

Experimental

Two 87 nucleotide long complementary oligonucleotides encoding for the repeat segment of DN3 were synthesized on a Milligen/Biosearch Cyclone Plus DNA synthesizer. These oligonucleotides were annealed to form an 87 base pair oligonucleotide, 1, which possesses interrupted palindromic 5' and 3' ends. Thus allowing for self-ligation without the formation of inverted repeat segments. Via self ligation concatemers of the DNA segment encoding DN3 may be generated. These interrupted palindromic ends are also compatible with the overhangs generated by the restriction digest of an appropriate DNA sequence by BstEII. Two additional 57 base long pieces of complementary DNA were also synthesized and annealed by the before mentioned methods. This DNA segment, 2, contains a 5' overhang complementary to a BamHI digest and a 3' overhang complementary to a SacI digest. This oligonucleotide also contains a 24 bp segment containing the ribosomal binding site from the pKK233-2 "ATG" expression vector positioned 5' to the translational start codon, ATG. A BstEII restriction site has been engineered into the middle of 2 and

incorporates a portion of the translation start codon in its' recognition sequence. The DNA segment 2 is ligated into the high copy number pUC19 vector. The resulting ligation mixture was then used to transform the bacterial strain DH5aF. Transformants were screened using a colony hybridization method using ^{32}-P labeled single stranded DNA complementary to 2 as a probe. Positive clones were analyzed by restriction digestion and a clone designated pUC19.mod was chosen for further cloning procedures. The vector pUC19.mod was then digested with BstEII and a monomer of 1 ligated into this vector. Positive transformants were again screened using colony hybridization the probe was an ^{32}-P labeled segment of DNA complementary to the segment 1. At this time the desired clone containing a single copy of 1 was excised from the pUC19.mod vector by restriction digestion with BamHI and Sac I. The resulting fragment was then ligated into the expression vector pNH8a, purchased from Stratagene. This reaction mixture was then used to transform the bacterial stain DH5aF. Positive transformants were screened by again using ^{32}-P labeled DNA complementary to 1. Positive transformants were confirmed by restriction digestion. Plasmid DNA was isolated from one of the positive transformants and used to transform the host bacterial strain D1210HP, necessary for the expression of the pNH8a clone containing a single copy of 1. This expression vector pNH8a possesses a novel induction system which requires not only induction with isopropyl-1-thio-ß-D-galactoside but also a short heat pulse at 42°C for 10 min.

Results and Discussion

At this time clones of the pUC19.mod vector have been isolated containing from one to approximately 13 repeats of the DNA segment 1. The sequence of the pUC19.mod clone containing only one segment of 1 as

well as the DNA segment 2 has been confirmed via cycle sequencing. No aberrations or adulterations in the sequence of this clone were found. We are currently in the process of confirming the sequence of those clones which contain multiple copies of the DNA segment 1. At this time analysis of the primary cell lysates from the induction of the pNH8a clone containing a single copy of 1 via SDS-page is underway. Interim results and developments as well as a more detailed explanation of the design and cloning strategies for DN3 will be discussed.

Acknowledgements

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Rheological and Photophysical Investigation of Domain-Forming Hydrophobic Polyelectrolytes Based on Sodium 11-Acrylamidoundecanoate

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Introduction

Our research group continues to investigate the synthesis and characterization of hydrophobically modified water-soluble polymers. Acrylamide (AM) has been copolymerized with sodium 11-acrylamidoundecanoate (SA) utilizing monomer feed levels of 1, 5, and 10 mole % SA. SA is a water-soluble surfactant which polymerizes via a free radical, micellar mechanism. Synthesis of these copolymers, denoted the AMSA series, has been reported previously with monomer feed levels of ≥ 30 mole %, high conversion in short periods of time (< 1 hr.), and high molecular weight ($\sim 10^6$ g/mol)[1,2]. Also characteristic of these systems is an enhanced radius of gyration with increasing AM incorporation. Our goal is the development of a pH and salt-responsive system utilizing lower SA content (≤ 10 mole %); at SA incorporations higher than 10 %, protonation of the carboxylate groups at lowered pH effects water-insolubility. The properties of such a copolymer system may lead to applicability in drag reduction, enhanced oil recovery, flocculation of waste water, controlled release of pharmaceuticals and pesticides, and the rheology modification of coatings and personal care products.

Experimental

Reagents

The general procedure for SA monomer and AMSA copolymers followed by Gan et al.[1,2] was utilized. All materials, except where noted, were obtained from Aldrich Chemical Company and used as received. Acrylamide (Aldrich, electrophoresis grade) was recrystallized twice from acetone, and pyrene was recrystallized once from absolute ethanol. Deionized water was used for monomer and polymer syntheses, and also for viscosity and fluorescence measurements.

Aqueous polymer solutions were prepared gravimetrically. Saline polymer solutions were prepared by adding a constant volume of sodium chloride stock solution to a set weight of aqueous polymer solution.

To introduce pyrene probe to polymer solution, 1.0 ul of methanolic 10^{-2} M pyrene was added to 10 ml polymer solution to give a constant probe concentration of 1.0 uM. The solutions were then allowed to equilibrate overnight on an orbital shaker.

Instrumentation

Viscosities were measured with a Contraves LS-30 rheometer, at a temperature of 25°C and shear rate of 6.0 sec^{-1}.

Fluorescence emission spectra were recorded with a Spex Fluorolog-2 fluorescence spectrophotometer equipped with a DM3000F data system and 450 W XBO high pressure xenon lamp as excitation source. Excitation slit width was set at 1.0 mm, and emission slit width varied from 1.0 to 2.0 mm, depending upon sample concentration. Samples were excited at 336 nm and emission monitored at 372 and 382 nm (1st and 3rd vibronic bands, respectively). Right angle geometry was employed.

Results and Discussion

Viscosity Studies

The apparent viscosities of the AMSA series (Figure 1) in deionized water, shown in Figure 2, do not reflect any appreciable intermolecular association or overlap. In the case of AMSA-5 and AMSA-10, none is seen below 1.0 g/dl. However, an upwards curvature is seen in the case of AMSA-1; the response approximates that of high molecular weight polyacrylamide ((PAM) 5-6 x 10^6 g/mol). This phenomenon suggests a chain-entanglement-driven response, rather than one arising from hydrophobic association. These systems tend toward intramolecular association[1,2] and viscosity studies indicate no exception for low-SA incorporation. With increasing SA content, the viscosity response lessens; Gan et al.[2] reported a direct proportionality between mole % SA and molecular weight. With increasing SA incorporation, viscosities in deionized water increase, but in all cases,

no intermolecular association is readily apparent. Polymerization proceeds by micellar and homogeneous mechanisms. When a radical chain end is propagating in aqueous solution, where AM is the major constituent, long runs of AM along the polymer chain result; however, when a radical encounters a mixed SA-rich micelle a "block" of SA is incorporated into the chain. These are not true blocks, but regions along the coil where SA predominates. As mole % AM is increased in the monomer feed, the average spacing between SA units and "blocks" increases. Arising from this is a more open conformation and a higher A_2 value and radius of gyration[2].

As reported by Gan et al., AMSA copolymers demonstrate a pronounced polyelectrolyte effect (Figure 3). As SA content increases, the polyelectrolyte effect strengthens. Reduced viscosity decreases with increasing polymer concentration. Shielding of coulombic repulsions due to the addition of salt reduces the hydrodynamic volume of AMSA polymers and Figure 4 illustrates this. AMSA-1 behaves similarly to PAM, but AMSA-5 and AMSA-10 have experienced a drastic viscosity decrease which is indicative of a highly collapsed conformation. We have also found that protonation of carboxylate groups by decreasing pH results in viscosity decrease. Essentially, diminishment of coulombic repulsions through shielding or elimination further collapses the polymer coil.

Photophysical Studies

Rheological studies alone cannot fully describe the solution behavior of such systems. A microscopic analysis in conjunction with a bulk, macroscopic method such as viscosity measurement, leads to a clearer understanding of a polymer's solution properties. Photophysical studies serve this purpose; fluorescence and absorbance experiments are highly sensitive and describe phenomena on a molecular level. To probe the hydrophobicity of the microenvironment, pyrene is added to AMSA solutions and solubilized by hydrophobic domains formed by association of SA units. Pyrene has previously been utilized to assess microdomain polarity[3] and aggregation number of hydrophobic groups[4] of intramolecularly associating water-soluble polymers. The ratio of the third vibronic band to the first, symmetry-forbidden emission band (I_3/I_1) is an effective gauge of the environmental polarity pyrene is experiencing. This ratio is inversely proportional to polarity. In deionized water (Figure 5) I_3/I_1 values increase to a maximum, then drop off. The minimum I_3/I_1 values approximate that of pyrene in water, reflective of an open, hydrated microenvironment. The profile suggests a micellization process. Maxima occur where the concentration of SA mer units in a given polymer roughly equals the critical micelle concentration (CMC) of SA monomer. The rise would result from association of pyrene with an increasing number of hydrophobes. When a sufficient number of hydrophobes are associated, micellar structures begin to form. Increasing electrostatic repulsions induce a highly fluid structure, more penetrable by solvent. At concentrations above "CMC", I_3/I_1 values drop off.

Conclusions

A series of water-soluble acrylamide copolymers with low incorporation of a hydrophobic anionic comonomer, sodium 11-acrylamidoundecanoate, have been synthesized. These copolymers exhibit a collapsed conformation due to intramolecular hydrophobic association. The viscosity response of AMSA copolymers appears to be driven by chain entanglement, and is controlled by SA incorporation. Pyrene probe studies suggest that intramolecular associations are manifested as micellar structures along the polymer chain. AMSA copolymers covalently labelled with fluorescent moieties is currently underway. Photophysical studies of systems like these gives further insight into the microscopic solution behavior of polymers. Correlation of macroscopic analysis with microscopic examination results in development of structure-property relationships, and may ultimately allow development of water-soluble materials targeted for specific applications.

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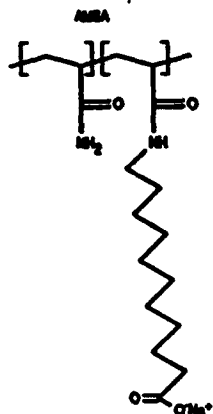4.   Chu, D.; Thomas, J.K. *Macromolecules* 1987, *20*, 2153.
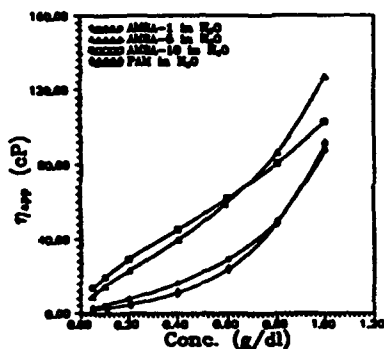
Figure 1. AMSA Copolymer Series.

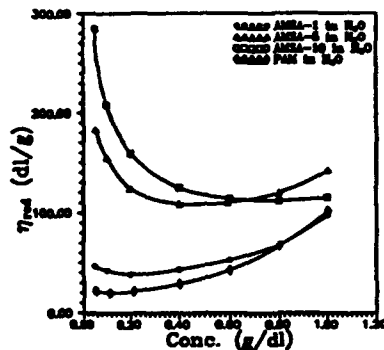Figure 2. Apparent Viscosity vs. Polymer Concentration in H_2O.
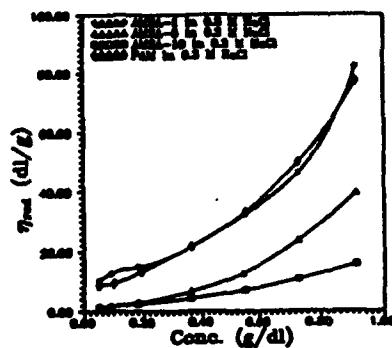

Figure 3. Reduced Viscosity vs. Polymer Concentration in H_2O.

Figure 4. Reduced Viscosity vs. Polymer Concentration in 0.3 M NaCl.
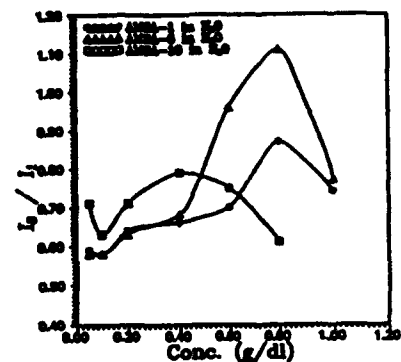
Figure 5. I_3/I_1 vs. Polymer Concentration in H_2O.

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Cloning and Expression of *Manduca sexta* Apolipophorin -III: A Model Protein for Removal of Hydrophobic Contaminants from Water

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Introduction

Hydrophobic foulants are largely responsible for the contamination of environmental water systems; therefore, the need exists for a way to safely and efficiently remove these foulants from water. Remediation of these molecules may be possible through the use of amphiphilic polymeric molecules which form micelles in solution. Both naturally-occurring and synthetic, micelle-forming polymers have been shown to sequester hydrophobic materials.[1-3] Many proteins, for example, associate *in vivo* to solubilize and transport oil containing particles.

One such protein is the apolipophorin-III protein (ApoLp-III) in the insect, *Manduca sexta*. In the insect, the protein reversibly associates with hydrophobic lipophorin particles consisting of phospholipids and diacylglycerols. The protein stabilizes the particles and allows them to load hydrophobic material. In addition, it facilitates the transport of these particles through a primarily aqueous insect hemolymph.[4]

The reversible association of the protein with the lipophorin particle makes it an ideal polymer for phase transfer of hydrocarbons from aqueous systems. In the conceptual scheme in figure 1, such a polymer is depicted as existing in a micellar form in water. Upon introduction of a hydrocarbon foulant to the system, the polymer would phase transfer the foulant into its interior. The filled micelles might then aggregate and flocculate, providing an easy means of collecting them. Perhaps a pH change would allow separation of the collected polymer from the foulant, permitting the polymer to be recycled.

Although the interactions of ApoLp-III with lipids have been extensively studied,[5-6] the interactions with hydrocarbons have not yet been explored. Recombinant synthesis of ApoLp-III in *E. coli* should facilitate production of large amounts of protein, and should allow site specific changes to be made in the protein sequence. With the ability to make such changes comes the potential to enhance the interaction of the protein with hydrocarbons, and to introduce pH responsive release of the hydrocarbons from the protein.

Experimental

Materials and Methods

Cloning vectors, PCR-II, and pMal-c2 were obtained from Invitrogen Corp. and New England Biolabs (NEB), respectively. All enzymes were obtained from NEB. Transformations were according to the Hanahan procedure.[7] Denaturing polyacrylamide gel electrophoresis (SDS-PAGE) was performed using equipment and reagents from Bio-

Rad as well as Sigma Chemical Company (SDS). Gels were stained using Coomassie Brilliant Blue and subsequently silver stained (Bio-Rad). Protein quantitation was accomplished using the BCA kit from Pierce. Immunoblots were performed with Bio-Rad equipment and reagents. Immunoblot development was accomplished using NBT/PCIB reagents from Pierce. Amylose resin and Factor χ_A protease were obtained from New England Biolabs.

Cloning of the *apolp-III* gene

The entire coding region of the *apolp-III* gene was isolated from a λ phage cDNA library (gift of Dr. Robert Ryan , University of Alberta, Edmonton) using the polymerase chain reaction (PCR) and PCR primers flanking the gene. The PCR product was cloned into pCR-II. INVαF' *E. coli* cells were transformed, according to the with the recombinant pCR-II vector containing the *apolp-III* gene. DNA from positive colonies was isolated and analyzed by restriction digestion with Eco RI. The *apolp-III* gene was recloned into the pMal-c2 fusion protein expression vector. TB1 *E. coli* cells were transformed with the pMal-c2/*apolp-III* recombinant. Positive colonies were identified by colony hybridization to a radiolabeled probe of the *apolp-III* gene, and DNA from them analyzed by restriction digestion with Xmn I. This DNA was sequenced using the BST kit (Bio-Rad) with primers made by DNA Int'l.

Expression and Purification of ApoLp-III

ApoLp-III expression from recombinantly transformed TB1 cells was induced by the addition of IPTG (isopropylthio-β-D-galactoside). The cells were lysed and separated into soluble and insoluble fractions which were analyzed by SDS-PAGE, column chromatography, and immunoreactivity. Antibodies to ApoLp-III (protein gift Dr. Robert Ryan) were raised in a New Zealand white doe.

Results and Discussion

Analysis of DNA in positive colonies revealed that the entire coding region of the apolp-III gene had been cloned intact and in the proper orientation. Expression of TB1 cells transformed with recombinant (pMalc2/*apolp-III*) DNA yielded MBP-ApoLp-III as one of the protein products. The presence of the fusion protein in induced recombinant TB1 cells was identified by immunoblotting with rabbit antibodies against MBP, as well as with rabbit antibodies against ApoLp-III.

Lysed recombinant TB1 cells were separated into soluble and insoluble fractions. Purification of the fusion protein from other proteins in the soluble matter proceeded via column chromatography on amylose resin. The fusion protein was found to elute, with 10 mM maltose, shortly after the void volume of the column. Fractions containing the fusion protein were pooled and concentrated with a microcon 10 (Amicon). Figure 2 shows that this chromatography step provided an excellent means of purification for the fusion protein.

Since the native ApoLp-III is our primary target, it was necessary to remove the MBP portion of the fusion protein. Factor χ_A protease was used to cleave MBP from recombinant ApoLp-III. As can be seen in Figures 2 and 3, the digestion was only marginally successful, yielding products which were not immunoreactive to antibodies against ApoLp-III

and which did not correspond to either the reported molecular weight of MBP or ApoLp-III.

Conclusions

Recombinant production of ApoLp-III opens a wide range of possibilities for the study of the interaction of this protein with hydrocarbons. Recombinant synthesis of the protein provides a means whereby the protein can be produced in large quantities in bacteria and can be site specifically changed to enable tailoring of the protein for enhanced hydrophobe sequestration as well as for pH responsiveness. Future work will utilize light scattering and photophysical techniques to probe domain organization and sequestration ability of the recombinantly produced ApoLp-III and its precisely mutated analogs.

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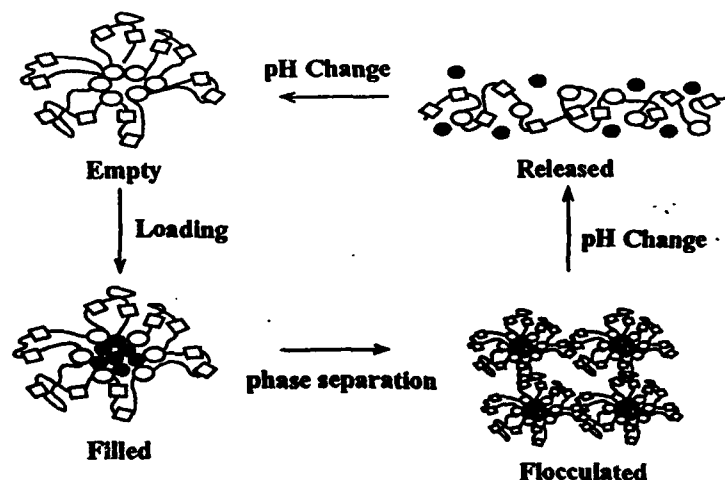7.   Hanahan; et al.; J. Mol. Biol., 1983, 166, 657.

Figure 1. Conceptual model of an amphipathic polymer for sequestration of organic foulants in water. O hydrophobe, □ hydrophile, ● organic foulant.
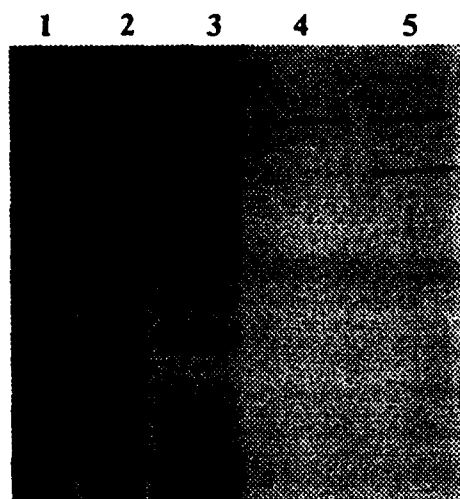
Figure 2. 12% SDS-PAGE, silver stained. Lanes: 1- uninduced TB1 cells with recombinant DNA; 2- cells 2 hours after induction; 3- soluble cell proteins; 4- fractions containing fusion protein after affinity chromatography; 5- fusion protein after Factor χ_A cleavage.
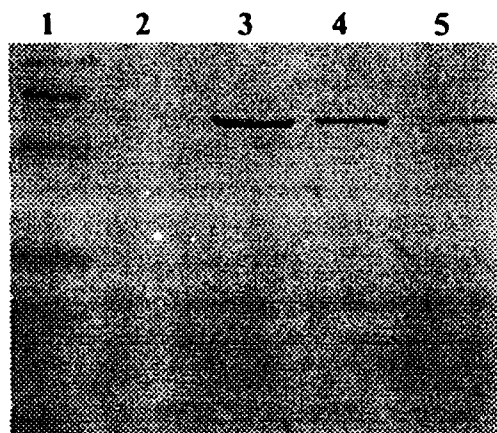

Figure 3. Immunoblot with antibodies against ApoLp-III Lanes: 1- prestained molecular weight markers (Bio-Rad); 2- uninduced TB1 cells with recombinant DNA; 3- soluble cell proteins from induced cells containing recombinant DNA; 4- pooled fractions containing fusion protein after affinity chromatography; 5- fusion protein after Factor χ_A cleavage

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The Use of Recombinant DNA Technology to Produce Water-Soluble Polymers

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Introduction

Water-soluble polymers are utilized in a wide variety of commercial applications. Polymer properties may be attributed to two factors: 1)the physical nature of the constituent monomers and 2) their sequential placement within the polymer chain. Hydrophobic tailoring of polyelectrolytes allows reversible domain formation which can be "triggered" by salt or pH change.[1,2,3,4,5] These systems, described as polymeric micelles, possess many advantages over small molecule surfactants and are suitable for applications requiring the phase transfer of nonpolar substances in aqueous media.

Most synthetic polymerization schemes currently used to produce many hydrophobically modified polyelectrolyes preclude the precise control of polymer microstructure. However, biological systems can produce polymers with a precise polymer microstructure. The tailoring of such systems has been facilitated by the advent of recombinant DNA technology.

Many proteins and polypeptides possess properties and functionalities comparable to synthetic polymer systems, yet the production of polypeptide based polymers for materials science applications via this technology remains relatively unexplored. Materials produced in this manner possess a well defined microstructure allowing for the tailoring of material properties for specific applications. Additional attributes include biodegradability and/or biocompatability.

Model System and Polypeptide Design

At this time, our research is focused on utilizing designed polypeptides and recombinantly engineered proteins to produce polymers capable of sequestering nonpolar molecules in response to changes in pH. This concept is illustrated in figure 1. The protein apolipophorin-III (apoLp-III) is an example of a -helical bundle protein which not only contains an interior hydrophobic domain, but has also been shown to interact with nonpolar fatty acid acyl chains.[6,7,8,9] ApoLp-III from the insect *Lucosta migratoria* has recently been crystallized and the three dimensional structure of this protein determined. This structure consists of five amphipathic α-helices connected by short loops. The nature of these helices is such that the nonpolar face of the helices are oriented towards the inside of the protein, resulting in the formation of a hydrophobic microdomain. This protein has been shown to associated and assist in the stabilization of lipoprotein particles *in vivo* as well as lipid vesicles *in vitro*. Figure 2.

We are currently involved in the *de novo* design and recombinant expression of a analogous polypeptide (DNL3), predicted to contain an amphipathic α-helix followed by an acidic "turn" or hinge region. Figure 3. This polypeptide sequence incorporates a variety of specific structural elements designed to enhance its performance as a pH-responsive phase transfer agent. A high helical propensity is demonstrated by most of the amino acids utilized for the α-helical segment of DNL3. The placement of nonpolar leucine residues with a periodicity corresponding to the **a,d** and **e** positions of a heptad repeat (e.g. **a,b,c,d,e,f,g**) ensures the formation of a distinctly nonpolar face within the α-helical segment of DNL3.[10,11] The overall hydrophobic moment of the α-helical segment allows it be classified as a "surface-seeking" amphiphile according to the method of Eisenberg.[12] Acidic and basic amino acids have been placed at the amino and carboxyl termini, respectively, in order to neutralize the macrodipole moment of the α-helical segments.[13,14,15,16] These amino acids serve to further stabilize by forming sidechain-to-backbone hydrogen bonds, resulting in the "capped" amino and carboxyl termini. Glutamic acid and lysine residues have also been positioned within the α-helical region of DNL3 to form intra-helical salt bridges. Asparagine residues have been incorporated into the α-helical region to relax the packing of adjacent α-helices. This is a preliminary attempt to moderate the packing of adjacent α-helices; this may be necessary to ensure that the nonpolar portions of the α-helices are free to interact with nonpolar materials.

The turn region of DNL3 is designed to form a flexible hinge which will allow the facile rearrangement of contiguous amphipathic α-helices in response to changes in the ionization state of the pendant carboxylic acid groups. Glycine residues have been incorporated into this sequence to impart flexibility as well as to serve as terminators of α-helical structures. Proline has also been incorporated into this structure to assist in the formation of a turn allowing the reversal of chain direction in the polypeptide chain.

Cloning and Expression Strategy

Currently the synthesis of artificial genetic elements is limited to oligonucleotides of approximately 100 bases in length. In order to produce polymers containing multiple repeats of the DNL3 amino acid sequence a method of polymerizing small nucleic acid monomers (81 base pairs) has been devised. This strategy is illustrated in figure 4

Experimental

Synthetic oligonucleotides were produced using a Milligen/Biosearch Cyclone Plus DNA synthesizer. Double stranded oligonucleotides encoding for one repeat of the DNL3 amino acid sequence were concatemerized using T4 DNA ligase (New England Biolabs). Resulting concatemers were cloned into the pT7-7 vector obtained from Dr. Stanley Tabor (Harvard Medical School), using standard methodologies. This vector was modified to

incorporate a BstEII restriction site as well as three downstream translational stop codons . Positive transformants were verified by DNA sequencing using the Sequenase system (United States Biochemicals). Expression of a positive clone resulted in the appearance of a unique protein band as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), using the method of Okajima. Amino acid analysis was performed by the W.M. Keck Foundation Biotechnology Laboratory (Yale University). Isoelectric focusing was performed using pH 3.0-7.0 isoelectric focusing gels from Novex. All other procedures were performed using standard methodologies.

Results and Discussion

A positive clone containing one repeat of the DNL3 amino acid sequence was identified using colony hybridization techniques. The nucleic acid sequence of this clone was confirmed using dideoxy sequencing techniques. Expression of this clone resulted in the appearance of a novel protein band (DNL3) found in the soluble fraction of the whole cell lysate. This band comprises 8.5% of the soluble fraction as determined by digitalization of the stained SDS-PAGE gel. The estimated molecular weight of this polypeptide is 3250 using SDS-PAGE with the appropriate molecular weight standards.

Initial purification of DN3L was effected using nondenaturing PAGE. The acidic nature of DN3L allows it to migrate in an electric field without the presence of SDS. The desired band was visualized and excised from the gel. DN3L was eluted from the crushed gel slice by soaking in a high salt buffer overnight at 42°C. Following dialysis, the protein was analyzed using SDS-PAGE. Two bands appeared in the lane containing the crush and soaked purified DN3L. The second band has a mobility corresponding to a molecular weight of 14.4 kDa.

An alternative means of purification involves precipitating most of the endogenous *E. coli* proteins with 80% ammonium sulfate, DN3L remains in the supernatant. Ammonium sulfate is removed by way of dialysis. The dialyzed sample is then purified using a DE-52 column eluted with a sodium chloride gradient. Samples purified in this manner also show two bands on SDS-PAGE. Amino acid compostional analysis of these samples indicate purity of greater then 96% (mol. percent).

Several attempts to perform N-terminal sequence analysis on DN3L have proven futile. Amino acid analysis of the PVDF membrane to which the peptide was transferred gave an amino acid composition in good agreement with what is expected. Table 1 The amino acid sequence of DN3L does not contain any amino acids that are prone to cyclizing with the N-terminus. Cleavage of amino methionine by CNBr resulted in the appearance of a third band on the SDS-PAGE gel. The molecular weight of this band is in good agreement with the molecular weight anticipated for a dimer of DN3L. The appearance of this band is may be due to the exposure of DN3L to formic acid during the CNBr cleavage. It is also possible that the removal of the

group/groups protecting the N-terminus has effected the aggregation properties of DN3L. N-terminal peptide sequencing, laser desorption mass spectroscopy and amino acid compositional analysis are currently underway with the CNBr cleaved DN3L.

Isoelectric focusing of DN3L gives an actual pI of 4.32 that is in good agreement with the theoretical pI of 4.03. DN3L migrates as a single band during isoelectric focusing, as detected by silver staining.

The conformation of DN3L in D_2O has been determined using FTIR. The presence of a strong peak at 1649.24 cm^{-1} correlates with the amide I peak of α-helical structures. Subsequently another peak at 1670cm^{-1} is indicative of β-turns. Thus confirming the presence of both helical and turn structures within DN3L in solution.

To date a peptide consisting of one turn-α-helix motifs has been expressed. The appearance and co-purification of a second band of higher molecular weight indicates the formation of DN3L aggregates. The molecular weight of this aggregate indicates that approximately 4 molecules of DN3L are present in the aggregate. The aggregate as well as the monomer (DN3L) both appear to be very resistant to the denaturing effects of SDS and temperature. The migrational behavior of these two bands is also conserved on PAGE gels containing 8.0 M urea. Analysis of DN3L by laser desorption mass spectroscopy has verified the molecular weight of DN3L 3,720 Daltons. Additional peaks were observed at 7,444, 11,178 and 14,900 Daltons, indicating various sized aggregates of DN3L.

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AN INVESTIGATION OF PLANT OLEOSINS AS POLYMERIC EMULSIFIERS.

Monica E. Tisack* and Robert Y. Lochhead, Dept. Polymer Science,
University of Southern Mississippi, Hattiesburg, MS 39406.

Introduction

The investigation of plant oleosins as polymeric emulsifiers was designed to study naturally occurring amphipathic polymers as alternatives to current small molecule surfactants and polymeric emulsifiers. The ultimate goal is the development of natural polymers capable of removing pollutants from waste discharge or inadvertent spills that occur in naval environments.

Emulsifiers are most commonly known as small molecule surfactants which serve to compatibilize two immiscible substances and provide a stable emulsion or suspension. Polymeric emulsifiers are less widely known, however possess several advantages over small molecule surfactants. Polymeric emulsifiers yield longer times of stabilization due to greater distances of repulsion between dispersed droplets, (because the polymer chains form loops and tails). Once applied to a substrate, and coalescence has occurred, the phase separation of the polymer requires large amounts of energy to re-emulsify. This is advantageous in applying herbicides and pesticides, where toxic run-off can occur due to the ease of re-emulsification by rainfall. Another advantage is that the weight percents needed for required stabilities is much less for polymers than for small molecules. Keeping this in mind, the study of oleosins, which are specific plant proteins, as emulsifiers is feasible for several reasons. The first being that such molecules are found in nature to exhibit desired behavior that is difficult to obtain synthetically. Another reason is that the oleosins are high molecular weight, therefore considered polymers. This allows all of the advantages of polymeric emulsifiers to be applied to oleosins. In addition, because these polymers are naturally found proteins, they have the added benefits of being an environmental source as well as having a more 'natural' degradation in our ecosystem. Lastly, their biological processes suggest that the proteins may be recyclable as emulsifiers, hence a zero-waste product.

Plants contain micellar particles, known as oil bodies, which function to compatibilize hydrophobic components within a water soluble medium. The micellar particles are made up of lipids, phospholipids, and proteins (see figure 1). The proteins, or oleosins, associate noncovalently with the surface of the micelles. This association indicates amphipathic character of the proteins that can be linked to molecular conformation. The oleosins of this study were obtained from soybean seeds. Preliminary studies have shown that soybean oleosins are interfacially active toward water/cyclohexane interfaces. These results were compared with phase diagram data in efforts to describe the feasibility of using such oleosins as primary emulsifiers. Also, effects of bacteria and protease inhibitors on interfacial activity are currently being evaluated.

Figure 1. Plant Oil Body

oil body   oleosin   phospholipid
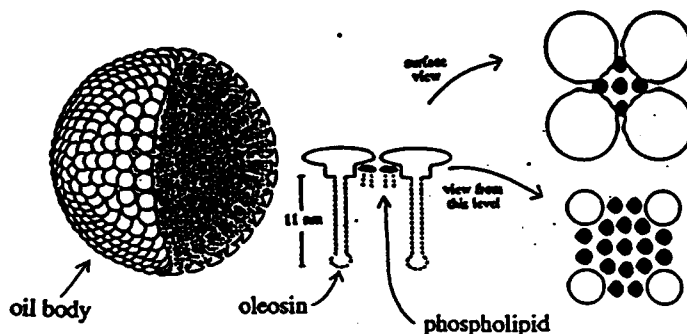
The soybean oleosins investigated contain approximately 223 amino acids, corresponding to a molecular weight of 24,000 g/mole. All oleosins possess three characteristic regions: a 40-60 amino acid N-terminus region, a 68-74 amino acid middle region which is totally hydrophobic, and a 33-40 amino acid C-terminus moiety. The N and C-terminus regions are amphipathic, with partial helicity. The center hydrophobic area was determined by Tzen (unpublished), to create an anti parallel β-strand 'finger' that penetrates the hydrophobic interior of the micellar particle. The previous features of protein conformation may be seen in Figure 2. (Huang)

Figure 2. Conformation of Oleosins
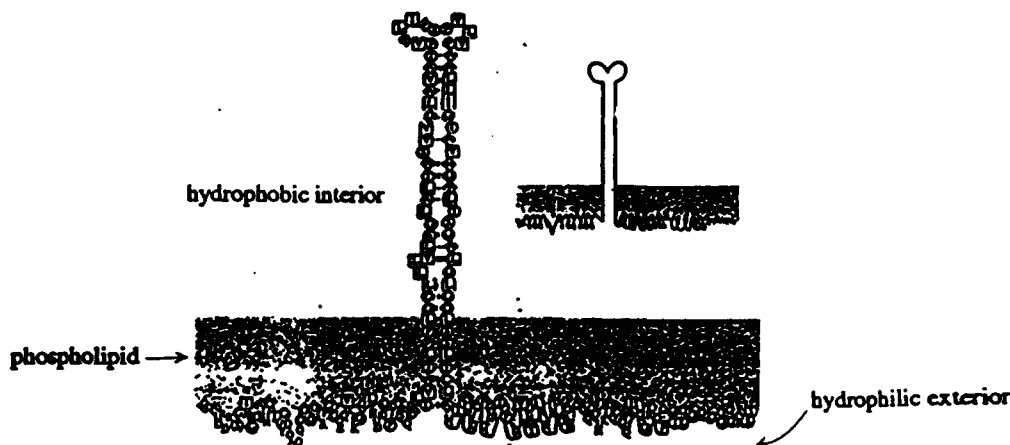
The primary objectives are to evaluate the oleosins as interfacially active substances and to determine the interfacial activity and emulsification abilities under various conditions. These objectives are approached by tensiometry experiments which allow the interfacial tension to be obtained over various oleosin concentrations. Once the interfacial trends have been obtained, (which elucidates the presence of oleosin at the oil/water interface), phase diagrams are created over the area of interest to determine the regions where stable emulsification takes place.

Experimental and Results

Interfacial tension measurements were carried out using a Kruss Processor Tensiometer K12 with the Du Nouy ring method. Results reported are within a standard deviation of 0.02 mN/M. Experiments were carried out at room temperature. All samples were prepared in glassware that had been cleaned with nitric acid and rinsed several times with double distilled water. All water used was double distilled. Oil used in these experiments was Fisher spectrophotometric grade cyclohexane. Oleosin used was extracted and purified by Xue Jian Bin of the Biochemistry department at the University of Southern Mississippi.

First, several interfacial experiments were carried out on aqueous suspensions of the soybean oleosin, (see figure 3.). Graph A represents an oleosin suspension sample of 0.02 g/L, and shows an interfacial tension decrease at the cyclohexane/water interface from approximately 48 mN/M to 42 mN/M (avg.). Graph B represents a second sample with a concentration of 0.025 g/L. The latter sample showed a decrease in interfacial tension of approximately 8 mN/M. These results indicate that oleosin is somewhat interfacially active under these conditions. Note that pH was unadjusted for both samples. Variances in runs within a given sample are not understood at this time, however possible contributors include heterogeneous dosing to the interface, oleosin degradation due to bacteria or enzymatic digestion, and/or denaturing of the protein.

Figure 3. Interfacial Tension Measurements with Varying Oleosin Concentrations
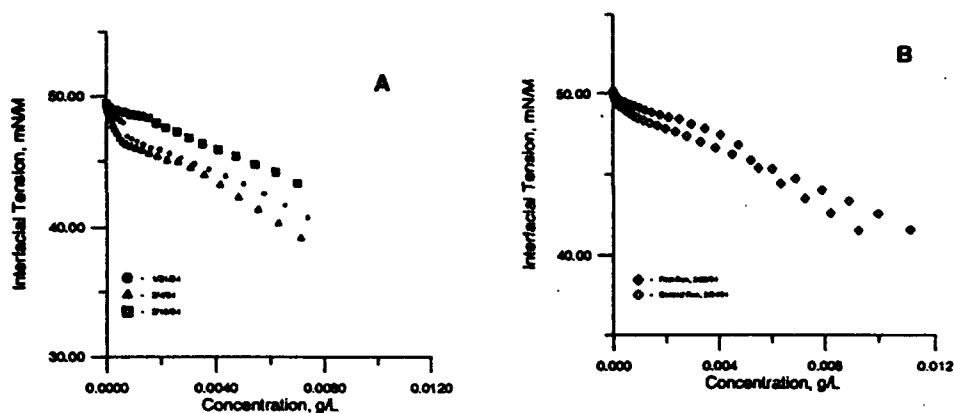

Due to the decrease in interfacial activity, phase diagram data was obtained for oleosin concentrations varying from 0.003 g/L to 0.021 g/L. For each concentration, individual samples were prepared with 1-10 wt% cyclohexane. Results are shown below in figure 4. Although coalescence rates varied, all samples had coalesced within one week.

Figure 4. Phase Behavior of Aqueous Soybean Oleosin with Cyclohexane
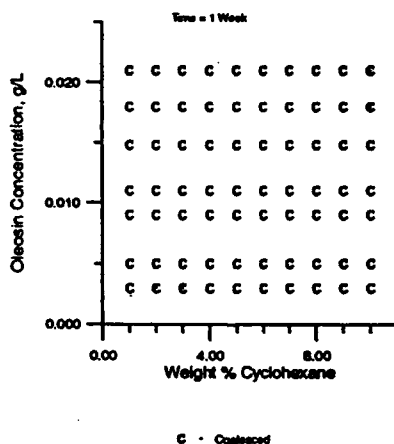
In attempts to determine whether degradation, (and its cause), of the oleosin was occurring, oleosin suspensions were treated with bacteria and protease inhibitors. In Figure 5, graph C represents an oleosin sample which was treated with 0.016 wt% Na Azide to prohibit bacteria growth. Again, reproducibility is in question; however the interfacial tension decreased 18 mN/M (avg.). Graph D corresponds to the oleosin suspension treated with 0.037 vol.% (total) of two protease inhibitors, 1:1 Phenyl Methyl Sulfonyl Fluoride and p-Toluene Sulfonyl Fluoride. The interfacial tension decrease was minimal, only 3 mN/M, however reproducibility is acceptable.

Figure 5. Interfacial Activity of Bacteria and Enzyme Treated Oleosin Suspensions
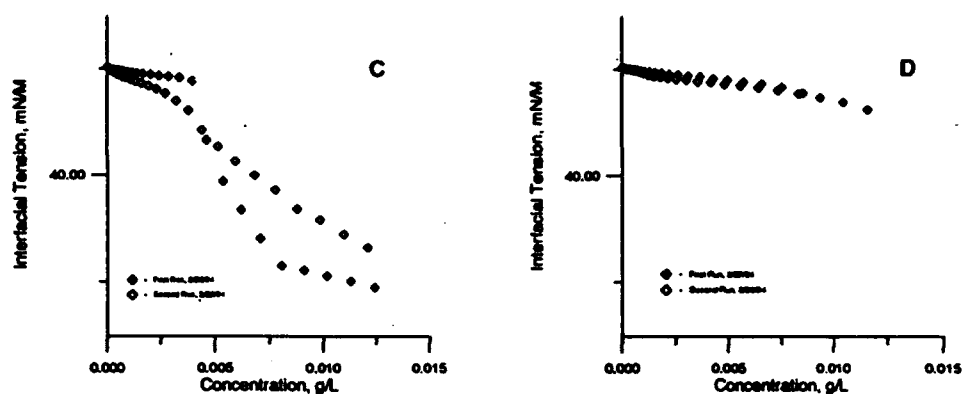
Conclusions and Future Work

Soybean oleosin shows slight interfacial activity toward the cyclohexane/water interface with no pH adjustments, and emulsification stability for concentrations between 0.003 and 0.021 g/L was unattainable. Varied reproducibility prompted the study of bacteria and protease inhibitors on interfacial activity. Results show that addition of bacteria inhibitor produced greater interfacial activity, and that addition of protease inhibitor leads to minimal activity. To conclusively determine a bacteria effect, pH studies should be evaluated, as addition of inhibitors may affect the natural 6.5 pH of the suspension.